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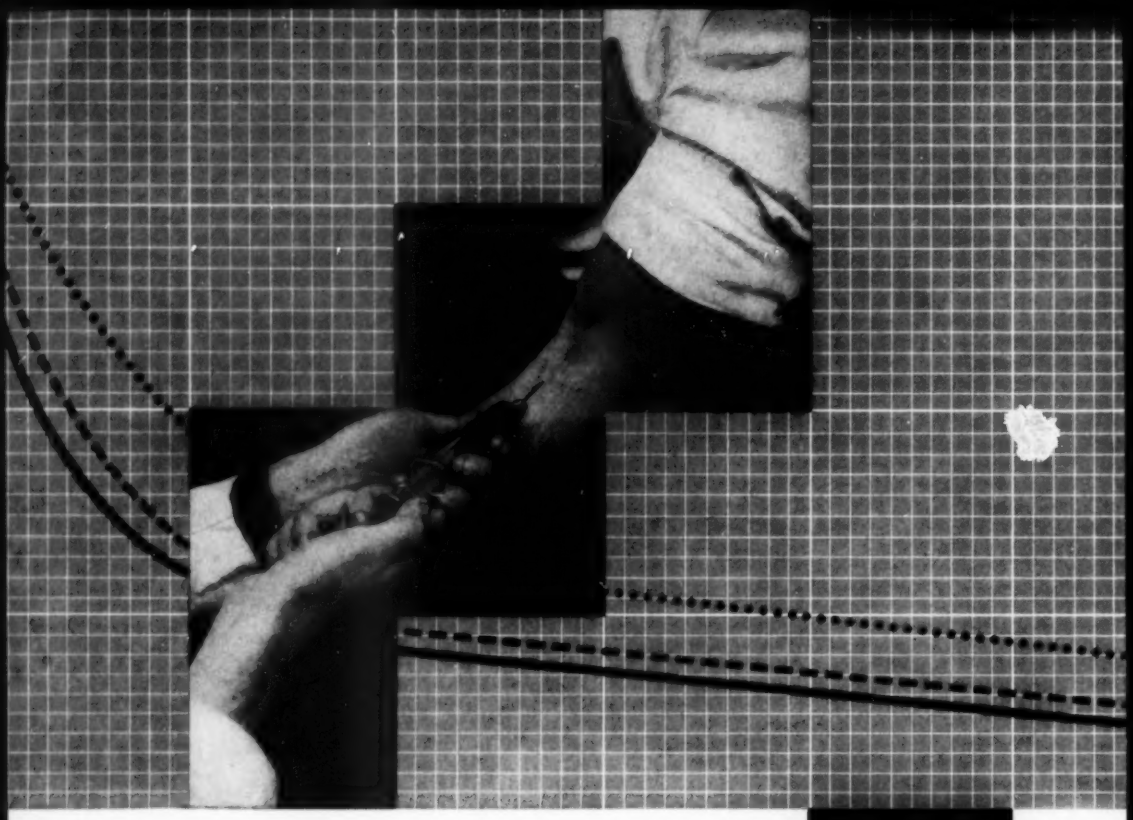

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2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951.
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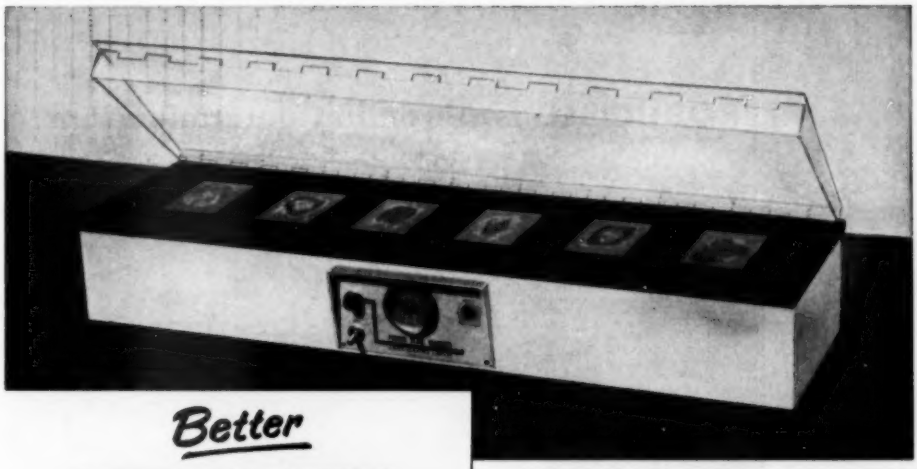

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ARCHIVES OF PATHOLOGY

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WEGENER'S GRANULOMATOSIS

Pathology and Review of the Literature

GABRIEL C. GODMAN, M.D.
AND
JACOB CHURG, M.D.
NEW YORK

KLINGER [1] in 1931 described a patient with severe destructive sinusitis, nephritis, and uremia in whom, at autopsy, splenic granulomata, arteritis, and glomerular lesions were found. Wegener,* recognizing in his three cases a distinctive syndrome, first characterized this complex clinically and anatomically. He held that it represented a unique variety of periarteritis nodosa, and since nasal and paranasal lesions predominated in his cases, he called the condition "rhinogenic granuloma." We have studied seven instances of this peculiar syndrome, as well as a number of similar and related disease patterns. In this communication we propose to describe the anatomic features and to review the pathologic anatomy and nosologic relationships of this disease.

SUMMARY OF CLINICAL FINDINGS

Detailed clinical data in our seven cases have been presented elsewhere.[4] There were five men and two females. Their ages varied from 12 to 50 years, with six patients falling between the ages of 35 and 50. The duration of illness was short in four patients—between 2 and 5 months; one patient lived 14 months and one 48 months. Interestingly enough, in the last case only minimal vascular lesions were found at autopsy. The seventh patient is still alive, nearly three years after the onset of symptoms, and has been maintained on cortisone and antibiotics.

The presenting clinical symptoms in six cases were sinusitis, subsequently associated with pulmonary disturbances, otitis, or arthritis. The skin and nervous system were frequently involved. All patients showed evidence of renal damage, such as albuminuria and hematuria; four died in uremia.

PATHOLOGICAL FINDINGS

Upper Respiratory Tract.—Lesions of the upper respiratory tract (nasal and oral cavities, paranasal sinuses, larynx, and trachea) were present in six of the seven patients. All six showed clinical evidence of sinusitis, and in four of these (G. O., R. K., J. M., G. M.) antemortem or postmortem specimens of nasal and paranasal mucosa were obtained for histological examination. In patients G. O.

From the Department of Surgery (Surgical Pathology), Columbia University College of Physicians and Surgeons (Dr. Godman), and the Division of Laboratories, Mount Sinai Hospital (Dr. Churg).

* References 2 and 3.

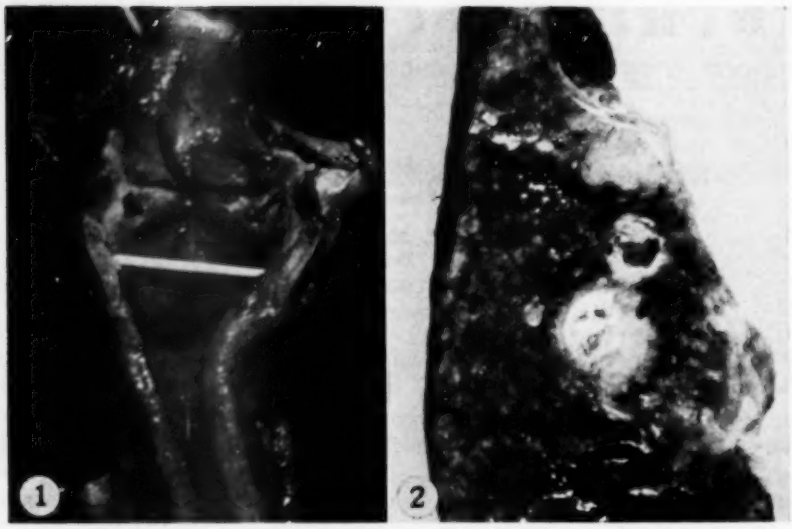

Fig. 1 (E. S.).—Larynx. Symmetrical ulcers of epiglottis and true vocal folds. The tracheal mucosa is thickened.

Fig. 2 (G. O.).—Lung. Discrete necrotic granulomatous masses, from 2 to 6 cm. in diameter. The advancing margins are made up of small, coalescent nodules.

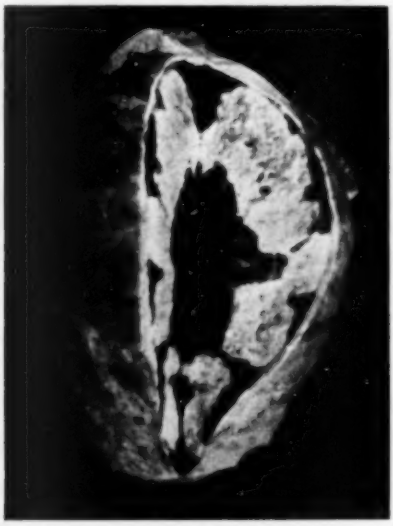

Fig. 3 (G. O.).—Spleen. Coagulative necrosis (infarction) and perisplenitis. There was thrombosis of the splenic vessels in this case.

and J. M. there were spectacular gross changes in all sinuses, with accumulation of thick pus, marked thickening of the mucosa, partial destruction of the bony confines, and extension of the process into the left temporal fossa.

Ulceration of the tongue was present in Patient R. K.; the ulcerative stomatitis of E. S. was apparently a part of generalized necrotizing dermatitis. Striking

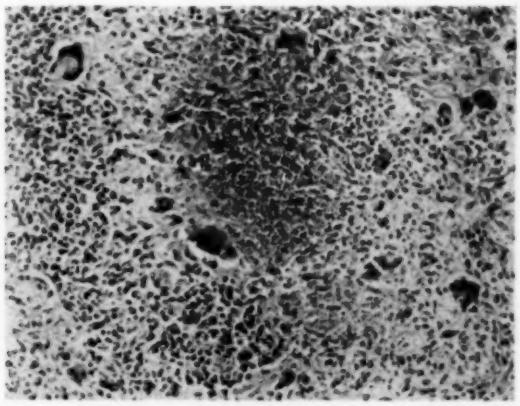

Fig. 4 (M. C.).—Lung. Necrotic focus with surrounding zone of inflamed granulation tissue and giant cells (granuloma). Hematoxylin and eosin; × 285.

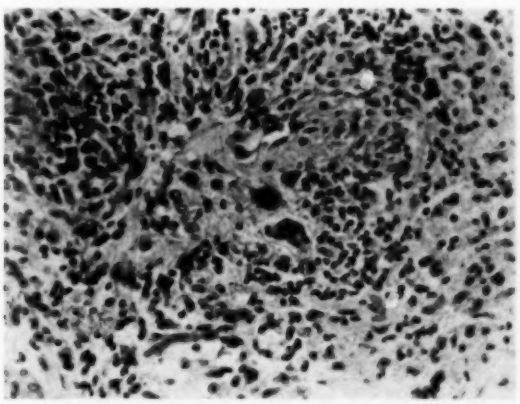

Fig. 5 (E. S.).—Lung. Organization and recurrent necrosis, showing the fibrillar character of such foci and the inflammatory response, including giant cells. Hematoxylin and eosin; × 233.

lesions of the larynx and trachea were present in E. S. (Fig. 1) and L. L. These consisted of edema, congestion, and extensive ulceration of the mucosa, particularly in the subglottic area and the vocal cords.

Microscopic examination showed very severe inflammation of the mucosa and the submucosa, with frequent involvement of small vessels in the area, both arteries and veins, by the inflammatory process (Fig. 6). The ulcerative process sometimes extended deep through the submucosa. In Case G. O. there was extensive necrosis

of tissue, with severe granulomatous reaction, many giant cells, fibrosis, and secondary necrosis of the fibrous tissue. These changes were of the type seen in the lungs (see next section). In a few cases, however, the sinusitis was not conspicuously granulomatous. Many small arteries in the involved areas were affected by severe necrotizing inflammation. Small granulomata with giant cells were also present in sections of the larynx and trachea of E. S.

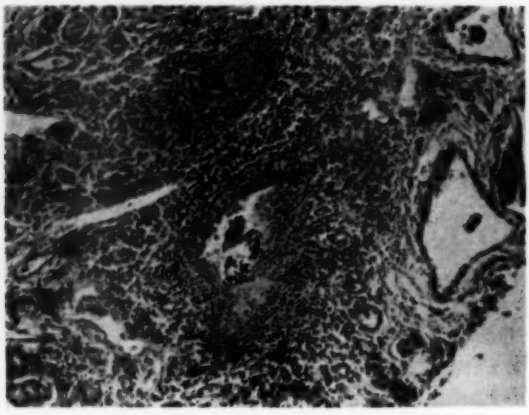

Fig. 6 (G. M.).—Arteritis, phlebitis, and periphlebitis in antral mucosa. Hematoxylin and eosin; × 100.

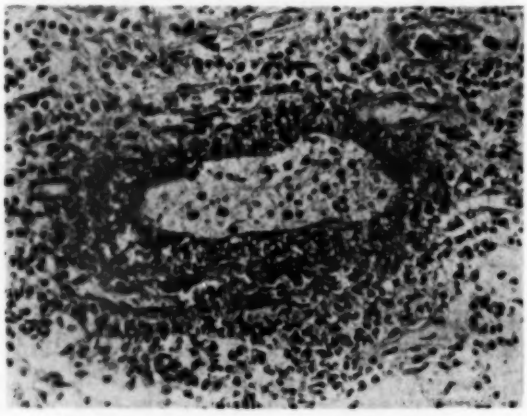

Fig. 7 (M. C.).—Small artery in kidney. "Fibrinoid" necrosis and inflammation. Hematoxylin and eosin; × 233.

Lungs.—The outstanding feature in all cases (six autopsies and one resection of the left lower lobe) was the presence of inflammatory masses within the pulmonary parenchyma of one or both lungs, measuring 0.5 to 5 cm. in diameter, and in one case, 9 by 8 by 3 cm. (Fig. 2). The masses were usually few in number and were sharply circumscribed. When close to the pleura, they often induced

deposition of fibrin on the surface. On section, they presented a gray, firm, rubbery periphery and a soft friable reddish-yellow to greenish-brown center. Branch bronchi were often seen entering the mass, where they were subject to compression of the lumen and ulceration of the mucosa. In one case (R. K.) the left main bronchus was affected in a similar manner. In some patients, notably E. S. and R. K., there were numerous lenticular ulcerations in the mucosa of both trachea and major

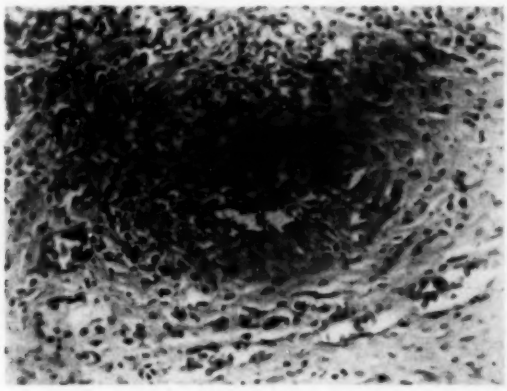

Fig. 8 (G. O.).—Small artery in nasal mucosa. Characteristic necrotizing arteritis, principally involving a sector adjacent to extravascular inflammation and necrosis. Hematoxylin and eosin; × 145.

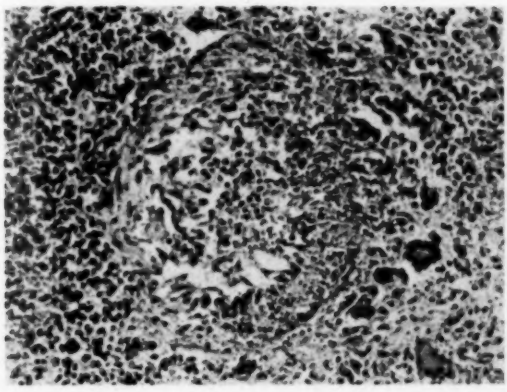

Fig. 9 (M. C.).—Small artery in lung. Sectoral necrosis and granulomatous inflammation, coextensive with granuloma in adjacent perivascular tissue. Hematoxylin and eosin; × 285.

bronchi. In addition, some patients also presented a number of well-circumscribed hemorrhagic infarcts, firm and dark red in color. Arteries supplying the infarcted areas were occluded by thrombi, but those running into the inflammatory masses were patent (Case G. O.). The pulmonary parenchyma between the masses was usually altered, being firm, poorly aerated, and red or gray.

Microscopically, the masses consisted of larger and smaller, often confluent, areas of necrosis surrounded by zones of granulation tissue (Fig. 4). Necrosis was

of an unusual type, in some areas obliterating all structural elements, and with much karyorrhexis, as seen in caseation, while elsewhere faint outlines of connective tissue fibers imparted to the necrotic areas a "fibrillar" appearance. The zone of granulation tissue contained many polymorphonuclear leucocytes, lymphocytes, plasma cells, and, rarely, eosinophiles. Case G. M., however, had more conspicuous

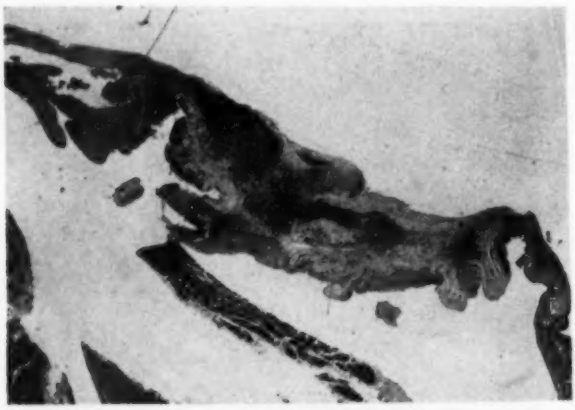

Fig. 10 (E. S.).—Mitral valve. Acute aseptic necrosis and inflammation of the central two-thirds of the valve leaflet, with extension into the chordae. Hematoxylin and eosin; × 10.

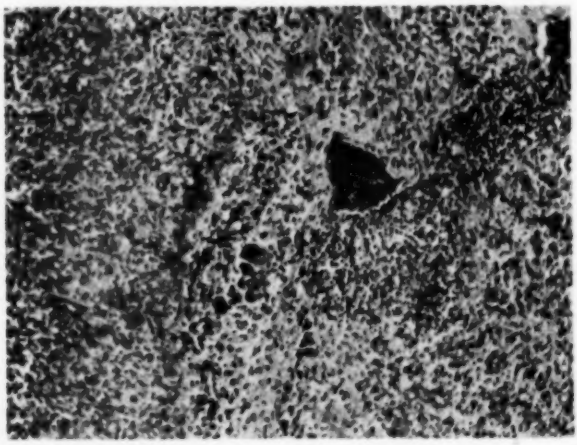

Fig. 11 (G. O.).—Spleen. Granulomatous trabecular arteritis and phlebitis, trabeculitis, and necrosis of the splenic parenchyma. Hematoxylin and eosin; × 33.

eosinophilia. Although fibroblasts along the edge of necrotic zones tended to form palisades, no true epithelioid cells were seen. The most conspicuous components were multinucleated giant cells, both of the foreign body and of the Langhans type. The sections give the definite impression of repeated peripheral organization of necrotic zones and subsequent extensions of necrosis into the fibrosed area (Fig. 5).

Granulation tissue with giant cells frequently extended into bronchi and bronchioles, replacing segments of the wall and narrowing the lumen. A similar focal process affected the trachea and bronchi in cases showing mucosal ulcers. Acute necrotizing arteritis of the type seen in periarteritis nodosa, with its sequelae, thrombosis of the lumen and fibrosis of the wall, was present in six of the seven cases. Smaller arteries and veins along the periphery of the necrotic masses showed sectoral inflammation and necrosis, particularly on the side of the vessel directed toward the mass. In the other case (L. L.), vascular lesions were of only the circumferential necrotizing type and were limited to one or two foci of acute inflammation and some fibrosed vessels.

The pulmonary parenchyma around and between the necrotic masses showed a variety of nonspecific changes—compression, exudation of polymorphonuclear leucocytes and fibrin, and collections of pigment-laden macrophages. The pleura immediately overlying a granulomatous mass presented fibrinous exudate on the

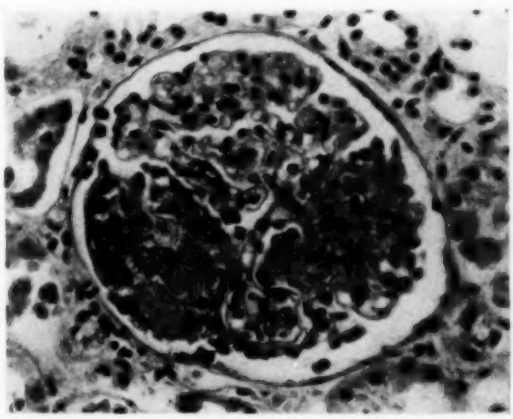

Fig. 12 (E. S.).—Kidney. Acute necrosis and thrombosis of capillary lobules of a glomerulus. Hematoxylin and eosin; × 295.

surface. Areas grossly diagnosed as infarcts showed a typical picture of necrosis and hemorrhage with a peripheral zone of congestion, but without the granulomatous response.

Kidneys.—These were moderately enlarged in most cases, and most showed fine punctate hemorrhages on the surface. There was a variable number of old and recent infarcts.

Microscopically, the most characteristic lesion, present in every autopsy case, was focal necrotizing glomerulitis, resembling that seen in subacute bacterial endocarditis and in certain forms of periarteritis nodosa. The number of affected glomeruli in any individual case varied from about 10% to over 70%. Usually only one lobe in each glomerulus, or even only a few loops, showed the change; but sometimes the glomerulus was almost completely involved, the necrotizing process streaking into the periglomerular zone beyond Bowman's capsule. The affected area presented deeply eosinophilic "fibrinoid" necrosis, with destruction of the capillary

loops and scanty polymorphonuclear exudation (Fig. 12). Healing was accompanied by hyalinization of the affected area, proliferation of the capsular epithelium, and capsular adhesions (Fig. 13). Foci of granulomatous inflammation with giant cells could be seen around some of the affected glomeruli in Patients R.K. and E.S. (Fig. 14), while similar small granulomata were present in the medullary connective tissue of Patient M. C.

In one patient (R. K.) most of the glomeruli showed also endothelial and epithelial proliferation, reminiscent of subacute glomerulonephritis.

Acute necrotizing and healed lesions of medium-sized and small renal arteries were present in five of the six autopsied cases.

Spleen.—The spleen was slightly to moderately enlarged and, as a rule, was covered with old and recent adhesions. In three of the six autopsied cases necrosis was present, in one (M. C.) in the form of multiple pinhead-sized, yellowish foci and in two as massive areas involving one-third of (G. O.) (Fig. 3) to nearly the

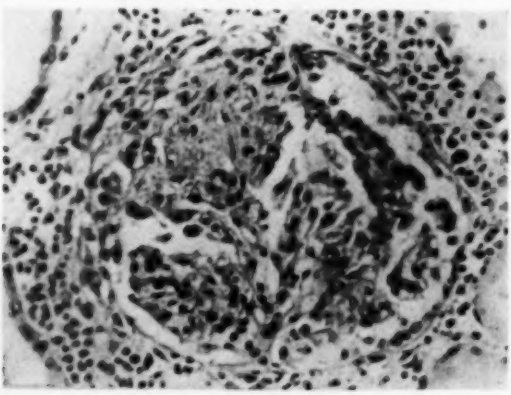

Fig. 13 (M. C.).—Kidney. Healing and healed focal glomerulitis, showing a fibrotic and adherent lobule and epithelial "crescent" formation of Bowman's capsule. Hematoxylin and eosin; × 265.

entire spleen (E. S.). In Case G. O. the splenic artery and vein, as well as the portal and mesenteric veins, were thrombosed.

Microscopic lesions were found in the three cases mentioned above and also in Cases L. L. and G. M. The first-mentioned case (M. C.) showed only focal trabeculitis, with small areas of early necrosis and focal accumulation of polymorphonuclear leucocytes. Trabecular lesions were much more prominent in the other four cases, in which connective tissue, trabecular arteries, and even veins were involved by acute or healing necrotizing processes, with epithelioid and giant cell reaction (Fig. 11). Follicular arteries were affected in a similar manner, particularly in E. S., causing the striking and massive splenic necrosis. The large infarct present in G. O., however, was probably due to involvement of the main splenic vessels.

Blood Vessels.—In addition to those in the respiratory tract, kidneys, and spleen, vascular lesions occurred in other organs, sometimes just at one or two sites, and in other cases (G. O., M. C., G. M.) being widely disseminated. The affected

arteries exhibited the classic changes of periarteritis nodosa, that is, acute inflammation of the wall, fibrinoid necrosis, thrombosis, and eventual healing by fibrosis. Vessel lesions of two kinds were found in the respiratory organs in our cases: inflammation and necrosis in sectors of both arteries and veins facing the extravascular lesions (Figs. 6, 8, and 9), and circumferential necrotizing arteritis of the more usual kind (Fig. 7). Involvement of the veins occurred also outside the respiratory tract, e. g., in the liver (L. L.) and spleen (M. C.).

An unusual lesion in case E. S., in the form of granulomatous inflammation with many giant cells, was present in a segment of the common iliac artery and vein and adjacent tissues.

Other Organs.—Small granulomatous nodules with giant cells, similar to those seen in the respiratory tract and the spleen, were found in the liver of Patient G. O., in the prostate and epididymis (Patients E. S. and M. C.), in the lymph nodes (M. C.), and elsewhere.

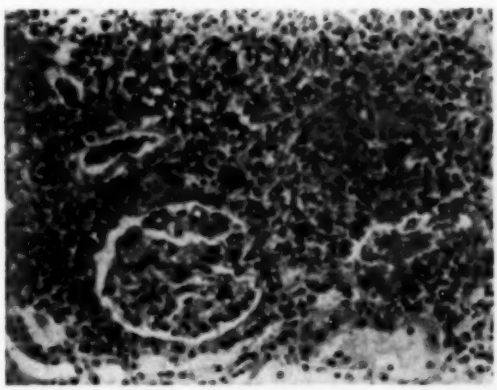

Fig. 14 (E. S.).—Kidney. Periglomerular granuloma in renal cortex. Hematoxylin and eosin; × 100.

Very unusual lesions of the mitral valve were present in Cases E. S. and G. M. In the former, most of the valve showed "fibrinoid" connective tissue damage and dense infiltration, with large mononuclear and polymorphonuclear cells throughout the entire thickness of the valve (Fig. 10). There were no vascular or inflammatory changes in the remainder of the heart. In Case G. M. there was superficial granulomatous inflammation with giant cells of the proximal part of the mitral valve. Similar granulomatous inflammation was present in the epicardium. Scattered in the myocardium of this case were foci of acute necrotizing coronary arteritis and small collections of mononuclear cells somewhat suggestive of Aschoff bodies.

Extensive coronary arteritis, recent and healing, with patchy myocardial necrosis, focal pericarditis, and endocardial mural thrombi was found in Case G. O. The valves showed no significant changes.

L. L., who died before the advent of sulfonamides and antibiotics, had bilateral mastoiditis and lateral sinus and jugular vein thrombosis with generalized septicemia (a hemolytic streptococcus).

COMMENT

Characterization.—The remarkable similarity of the pathological changes in all of the cases, as well as the many clinical features which they share, makes it probable that we are dealing with a peculiar and separate syndrome. It was Wegener † who clearly recognized a distinct entity with particular characteristics, which did not correspond to any of the known granulomata of specific etiology and which differed from other, more ordinary, forms of periarteritis nodosa.

The clinical course is that of a febrile disease, usually beginning in the respiratory tract, with severe progressive upper respiratory or pulmonary symptoms, often x-ray evidence of lung involvement, and more or less rapid decline, eventuating in uremia. The disease affects persons of both sexes and all ages, most of whom have no clinical stigmata of allergy or hypertension.

Anatomically, these cases which we have studied and regarded as typical of Wegener's granulomatosis were invariably characterized by concurrence of three pathological features:

1. Necrotizing granulomatous lesions in the upper air passages (nose, paranasal sinuses, nasopharynx, glottis, or adjacent regions) or in the lower respiratory tract (trachea, bronchi, lungs) or in both.

2. Generalized focal necrotizing vasculitis, involving both arteries and veins, almost always in the lungs, and more or less widely disseminated in other sites.

3. Glomerulitis, characterized by necrosis (and thrombosis) of loops or lobes of the capillary tuft, capsular adhesion, and evolution as a granulomatous lesion.

Together, these features are the identifying morbid characteristics, no one or two of which, in the absence of the others, serve to denominate the syndrome anatomically.

In addition, certain visceral lesions, while not a necessary part of this disease pattern, recur with a frequency which gives them particular interest. These are massive or multiple necroses in the spleen, usually associated with extensive central arteritis, splenic trabeculitis, and disseminated visceral granulomata. The lesions last mentioned, sometimes necrotizing, may be found particularly in the renal cortex (periglomerulitis), the male adnexal organs, the walls of blood vessels, and the spleen.

Defined by these clinicopathological criteria, necrotizing respiratory granulomatosis with angiitis (Wegener's granulomatosis) may be somewhat summarily segregated from the protean and miscellaneous category of vascular disease called "periarteritis nodosa," as an entity having clinically recognizable manifestations, a particular natural history, and clear anatomically diagnostic features.

With the 7 cases detailed herein, 29 cases are now known which conform to a clinicopathological pattern whose main characteristics have been outlined. These are listed in Tables 1 and 2. The case of Rössle,[17] Sandler and associates,[18] Cases 3 and 7 of Sweeney and Baggenstoss,[19] Cabot Case 37511,[20] Bohrod's case,[21] and Case 1 of Former [22] are also probably instances of this syndrome but either are atypical or are reported with insufficient detail to allow of evaluation.

Respiratory Tract: In the first published case the upper air passages were chiefly involved, and Wegener's designation focused attention on their priority.

† References 2 and 3.

TABLE 1.—*Summary of Seven Cases of Wegener's Granulomatosis*

Case	Age, Yr.	Sex	Clinical Features	Duration, Mo.	Upper Respiratory Tract Lesions			Lung Lesions			Kidney Lesions			Spleen Lesions			Other Granulomatous Lesions	Other Vascular Lesions
					Ulcerative	Granulomatous	Vascular	Ulcerative	Granulomatous	Vascular	Glomerular	Granulomatous	Vascular	Necrotic	Granulomatous	Vascular		
L. L.	43	M	Prolonged sinusitis, otitis, and laryngitis; terminal sepsis	48	+	+	+	+	+	+	−	−	−	−	−	+	—	Stomach
G. O.	39	M	Severe destructive sinusitis; terminal uremia	5	+	+	+	+	+	+	+	+	+	+	+	+	Liver	Widely disseminated
E. S.	50	M	Orchitis, epididymitis, arthritis, and dermatitis; terminal uremia	3	+	+	+	+	+	+	+	+	+	+	−	+	Prostate, epididymis, femoral vessels, and adjacent tissue	Epididymis
M. C.	34	M	Migratory arthritis and fever; terminal arthritis, rash, and uremia	14	−	−	−	+	+	+	+	+	+	−	−	+	Lymph nodes, prostate	Widely disseminated
R. K.	40	F	Severe sinusitis and pulmonary masses; sudden uremia	2	−	−	−	+	+	+	+	−	+	−	−	−	—	Ovary
G. M.	12	F	Severe intractable sinusitis, fever, and hematuria	1	+	+	+	+	+	+	+	−	+	+	+	+	Lymph node and perinodal tissue; pericardium	Heart, periadrenal tissue; perirenal tissue
J. M.	28	M	Severe destructive sinusitis and pulmonary granulomata; subacute course; evidence of renal damage; patient being maintained on cortisone and antibiotics	36*	+	− (Biopsy)	−	+	+ (Biopsy)	+	No data			No data			No data	No data

* At time of writing.

TABLE 2.—*Lesions in Twenty-Two Cases of Wegener's Granulomatosis Collected from the Literature*

Case	Reference	Age, Yr.	Sex	Upper Respiratory Tract Lesions			Lung Lesions			Kidney Lesions			Spleen Lesions			Other Granulomatous Lesions	Other Vascular Lesions
				Ulcerative	Granulomatous	Vascular	Necrotic	Granulomatous	Vascular	Glomerular	Granulomatous	Vascular	Necrotic	Granulomatous	Vascular		
1	1	70	M	+	+	+	−	−	+	+	−	+	+	+	−	−	
2	2 and 3	38	M	+	+	+	+	+	+	+	−	−		No data		−	Intestine, mesentery, gall bladder, testis, diaphragm
3	2 and 3	36	F	+	+	−	+	+	+	+	+	+	+	+	+	−	Intestine, mesentery, gall bladder, heart, diaphragm
4	2 and 3	33	F	+	+	+	−	−	+	+	−	+	+	+	+	−	−
5	5	44	M		No data		+	+	+	+	+	+	+	+	+	−	−
6	5	42	F		No data		+	+	+	+	+	+	+	−	+	−	
7	6	20	F	+	+	−	+	+	+	+	+	+	+	+	+	Liver, intestine, dura, lymph nodes	
8	7	35	F	−	−	−	+	+	+	+	+	+	+	+	+	−	Esophagus, pancreas, uterus, adrenals
9	8	67	F	+	+	+	−	−	−	+	+	+	−	−	−	−	Liver
10	9	38	M	+	+	−	+	+	+	+	+	+	+	−	+		Skin
11	9	50	F	−	−	−	+	+	+	+	+	+	−	+	+	Liver	Heart, adrenals, gall bladder
12	10	34	F		No data		+	+	+	+	+	−	+	+	+	−	Liver ....
13	10	62	F		No data		+	+	+	+	+	−	+	+	+	−	Liver
14	11	57	F	−	−	−	+	+	+	+	+	+	+	+	+	−	Liver
15	12	23	M	+	+	+	−	−	+	+	+	+	−	+	+	−	Tongue
16	13	69	F		No data		+	+	−	−	+	+	−	+	+	−	−
17	14	70	M	−	−	−	+	+	+	+	−	+	+	+	+	−	Stomach
18	13	41	F	+	No data		+	+	+	+	+	+	−	−	−	Liver	−
19	15	40	M	+	+	−	−	−	+	+	−	+	−	−	−	−	Heart, adrenal, nerves, muscle
20	15	51	M	+	−	−	+	−	+	+	−	+	+	+	+	−	Gastrointestinal tract, mesentery, liver, muscle
21	16	27	M	−	−	−	+	+	+	+	+	+	+	−	+	Bladder, prostate, lymph nodes, artery	Gastrointestinal tract, bladder
22	16	54	M	+	−	−	+	+	+	+	+	−	+	−	−	−	−

Subsequent authors,‡ dealing with cases in which respiratory tract lesions were apparently only pulmonic, correctly identified them with those cases in which nasal affection was outstanding. The respiratory tract lesions have been thought of by some to result from the disturbed circulation caused by primary vascular disease, in effect, infarcts.§ In our cases, and those of other authors,‖ there is clinical evidence of the priority of the respiratory granulomata in the course of the disease. The apparent lack of temporal and spatial relation of the granulomata to involved vessels in many cases, such as, for example, the patency of vessels entering the granulomata in our Case G. O., as contrasted with the occluded vessels serving the infarcted areas, makes it unlikely that the necrotizing granulomatous masses result from a prior vasculitis. On the other hand, it has been suggested,¶ because of an apparent distribution of involved vessels and affected vessel sectors about the granulomata, that the vessels are affected by centrifugal spread from antecedent necrotic extravascular foci. It would seem, then, that the respiratory granulomata as they finally appear represent the combined effects of a primary affection of the respiratory tissues and subsequent or concomitant regional angiitis.

Blood Vessels: The vascular lesions are in most cases acute, sometimes healing or healed; usually all stages are found. The sectoral necrosis and inflammation of some vessels adjacent to granulomatous masses are suggestive of an extension of the process from the adjacent tissues rather than of a primary angiitis (Figs. 8 and 9). Especially noteworthy were the early stages of arteritis and phlebitis seen particularly about the granulomata in some cases, in which extreme degrees of intimal edema and cellular infiltration of the vessel walls were found with little or no overt necrosis. The disseminated vascular lesions in respiratory and other organs were frankly necrotic, and presumably primary arteritides of usual appearance (Fig. 7). Vascular lesions may be very numerous and the outstanding feature of the disease, as in our case G. O., Wegener's first two cases, and Case 2 of Stratton and associates [15]; or very few, as in our Case R. K., and in others,# in which the granulomata dominated the scene. Granulomatous vasculitis, also present in some cases,* and notably in our Cases E. S. and G. M., may well be morphogenetically distinct from the acute necrotizing process. It probably represents the fortuitous occurrence in vessel walls of giant cell granulomata, having the same nature and significance as those occurring in other tissues.

Spleen: The splenic lesions encountered were especially remarkable for their extent. Qualitatively, they were of the kind (follicular arteriolitis, trabeculitis, capsulitis, follicular necrosis, granulomata, and multiple infarcts) reviewed by Ball and Davson [23] as manifestations of the "microscopic form" of periarteritis, or the so-called "hypersensitivity angiitis." † In some cases,‡ as in our Case 3, there was very striking massive or total splenic infarction, related to extensive follicular arteritis.

‡ References 5 and 9 through 11.
§ Reference 1 through 3 and 5, 6, 14, 17, and 19.
‖ References 9, 11 through 13, and 16.
¶ References 11 and 16.
# References 8, 13, and 16.
* References 5 through 7.
† References 23 through 27.
‡ References 1 through 3, 5, 6, and 16.

Kidney: Directly responsible for fatal issue in about 86% of cases, the renal lesions deserve particular mention. Like the vasculitis, the apparent anatomic ages of these lesions, when compared with the duration of the clinical course, indicate that they are the result of a continuing or recurrent process, which usually commences rather late in the disease, for in the majority of cases it is such a recurrent or initial attack upon the glomeruli which proves lethal. These necrotizing thrombotic glomerular lesions differ from the lesions of diffuse glomerulonephritis and are like those seen in association with subacute endocarditis, sepsis, and the microscopic form of periarteritis nodosa ("hypersensitivity" or "allergic" angiitis of Zeek §). The subacute forms bear certain resemblances to the experimental nephritides produced by protein or autoantibody injection.‖ The granulomatous glomerulonephritis and periglomerulitis, which Wegener ¶ regarded as unique, appear to be stages in the further evolution of the acute glomerular lesion and have been observed in other conditions.# Also prominent in the interstitium of the renal cortex in some cases,* as in our cases G. O., E. S., and M. C., were granulomata or looser infiltrations of the same kind as those found distributed in other sites.

Related and Similar Disease Patterns; Differential Diagnosis.—There are a number of disease entities with tissue changes very similar to those encountered in Wegener's granulomatosis, some of which are obviously related to it morphologically and pathogenetically. These group themselves into a compass from necrotizing and granulomatous processes without angiitis, through mixed forms, to vasculitis without granulomata.

1. Necrotizing and Granulomatous Processes: (*a*) Agnogenic progressive lethal granulomatous ulceration (granuloma gangraenescens) of the nose and facial midline. Few cases of this mutilating condition have been completely studied post mortem. In most of the autopsied cases the disease process was confined to facial structures, but in occasional cases reported under this heading, such as Case 3 of Williams [32] and Case 1 of Woodburn and Harris,[33] periarteritis nodosa and renal lesions were present. The indolent course of the more usual case of granuloma gangraenescens and the usual absence of generalized vascular, glomerular, and granulomatous lesions would seem to distinguish it from Wegener's granulomatosis, but it is probable that these conditions are closely related. We have studied a clinically typical case of granuloma gangraenescens of prolonged course in which at autopsy recent arteritic lesions were found. It may be that midline facial disease represents the localized manifestation of a disease which, becoming generalized, manifests itself as Wegener's syndrome.

(*b*) Specific infectious granulomatous diseases. Among the agents capable of exciting local tissue reactions of somewhat similar histologic appearances are those of leprosy, tuberculosis, syphilis, tularemia, glanders, blastomycosis, histoplasmosis, and lymphogranuloma venereum. None of these in its known forms is likely to reproduce a triad of morbid changes like that which characterizes Wegener's granu-

§ References 26 and 27.
‖ References 28 through 30.
¶ References 2 and 3.
# References 22 and 31.
* References 2, 3, and 6 through 10.

lomatosis. Diagnosis ultimately rests on demonstration of the etiologic agent or its effects, directly or serologically. In no case of Wegener's syndrome have such specific organisms been inculpated as pathogens.

(*c*) Boeck's sarcoid. This disease may sometimes involve vessel walls [34] and be distributed predominantly in those sites also affected in Wegener's syndrome. Distinction is made on the essentially non-necrotizing nature of the sarcoid lesion.

(*d*) Allergic granuloma and Loeffler's syndrome. Granulomatous lesions or looser infiltrates with eosinophiles occurring independently of vascular lesions are occasionally encountered in patients exhibiting undoubted clinical evidences of allergy (asthma, eosinophilia). In the case reported by Ehrlich and Romanoff [35] there were large circumscribed pulmonary granulomata. Disseminated infiltrations, often granulomatous, have been noted in other allergic states.† Closely related is Loeffler's syndrome, whose anatomic manifestations may vary from a diffuse eosinophilic pneumonitis without vasculitis [38] to necrotizing eosinophilic granulomata with pulmonary [39] or generalized arteritis.[40] It may be of interest that, while the rheumatic lung lesions of Masson, the so-called Masson bodies, have been found in a large proportion of cases of allergic granulomatosis, they have been uniformly absent in the cases here reported.‡ Although clearly related, these syndromes lack some clinical and pathological features of Wegener's granulomatosis. It may well be that certain of them represent milder or incomplete forms of a process fundamentally similar to that of Wegener's complex.

2. Angiitides: (*a*) Classic periarteritis nodosa. This designation should probably be reserved for the disease picture referred to in the older literature and by Zeek § in which medium-sized arteries are involved with formation of macroscopic nodules. Healing stages are usual; associated hypertension is common, and infarcts consequent on interrupted blood supply are the only consistent extravascular lesions.

(*b*) The microscopic form of periarteritis nodosa. Into this category fall perhaps half the cases of arteritis studied in recent years. Unequivocal clinical evidences of allergy are absent, although in some of these cases certain allergens, particularly synthetic drugs, would seem definitely to be incriminated. Their anatomic features have been detailed by Zeek § under the designation "hypersensitivity angiitis." Presumably, most of Zeek's cases are of the type which we include under the heading of the microscopic form of periarteritis. Extravascular lesions in the spleen [23] and kidney,‖ when present, are identical with those in our cases. The presence of necrotizing granulomata, and their extent and concentration in the respiratory system, serve to distinguish Wegener's granulomatosis from this form of arteritis, but it is plain that, as in the other listed instances, they are linked in some association.

3. Allergic Angiitis and Granulomatosis (Mixed Forms): As described by Churg and Strauss,[42] this group is characterized by a well-defined clinical syndrome of asthma, fever, and hypereosinophilia. Anatomically, it is representative of more widespread and aggressive tissue changes than the other categories listed and bears closest resemblance and relationship to Wegener's syndrome. However, no case

† References 24, 25, 36, and 37.

‡ Ehrlich, J. C.: Personal communication to the authors.

§ References 26 and 27.

‖ References 41 and 42.

of Wegener's granulomatosis known heretofore has had such definitive clinical stigmata of allergy as those encountered in the allergic angiitis syndrome. Wegener's syndrome further differs from allergic angiitis with granulomata in the predominant and aggressive granulomatous lesions of the respiratory tract in the former, much greater in extent and severity than in those cases detailed by Churg and Strauss.[43] That these disease patterns are, anatomically, instances in a continuous series is attested to by the instructive case of G. M., which must represent a link between these obviously related forms of disease.

The allergic history of this patient, the presence of eosinophiles in the exudates, and the diffuse interstitial myocarditis and endocarditis are clearly indicative of a disease of hypersensitivity. These features are generally absent in cases of Wegener's granulomatosis, but in this unusual case their combination with the characteristic picture of Wegener's syndrome reinforces the suspicion that it is closely related to allergic angiitis pathogenetically as well as morphologically.

ETIOLOGY AND PATHOGENESIS

The search for a specific infective agent, so far as it has been pursued,¶ has yielded only a mixed flora usual in the upper respiratory tract or as contaminants. It remains possible, although most unlikely, that a particular causative agent for Wegener's granulomatosis will be discovered. With the presently available material, much information may still be gained through careful consideration of clinical data, together with a comparative study of the pathological anatomy of Wegener's granulomatosis and analogous lesions, both human and experimental.

What we have been accustomed to call periarteritis nodosa is not a homogeneous grouping.# Indeed, necrotizing polyarteritis has been produced experimentally in a variety of ways—renal ischemia or insufficiency,* perinephritis,[48] precipitous increases in blood pressure,[49] and injections of corticosteroids [50] and of certain amine compounds.† Among these, sensitization for foreign protein ‡ is of particular pertinence. In human arteritis, possibly in one-third of all cases, there is clinicopathological evidence of hypersensitivity, inculpating particular antigens or haptens (sulfonamides,§ iodine,[57] thiourea,[58] diphenylhydantoin sodium [Dilantin],[59] and others), or an association of arteritis with known allergic states.‖ It thus seems certain that at least some cases of human polyarteritis are phenomena of anaphylactic hypersensitivity.

The granulomatous tissue response also occurs as a reaction to many diverse agents, both living and nonliving. Reduced to their common qualities, most of these causative agents of granulomata act either as foreign unabsorbable materials or as organisms which tend to provoke a high degree of sensitization and immunity to their products [61] or as both. While the production of sensitizing antibody is a characteristic feature in infectious granulomatous inflammation,¶ it cannot be

¶ References 1 through 3, 7, 16, and 32.
# References 26, 27, and 44.
* References 45 through 47.
† References 51 through 53.
‡ References 28, 54, and 55.
§ References 24, 25, and 54 through 56.
‖ References 39, 43, and 60.
¶ References 61 and 62.

insisted that the formation of granulomatous nodules necessarily depends upon the allergic state, at any rate, in tuberculosis.[63] It is, however, generally recognized that granulomatous inflammation, as well as tissue necrosis, may be found in hypersensitive states in a correlation which cannot be without significance.# Granulomata have also been produced experimentally in the course of eliciting the Arthus phenomenon.[66]

In the case of experimental glomerulonephritis, its production through antigen-antibody reactions * is too well known to require comment, and there are reasons for believing that the Löhlein lesion (focal necrotizing or thrombotic glomerulitis) may result from a similar mechanism of hypersensitiveness.

Forms of glomerulitis may occur, of course, in conditions in which hypersensitivity plays no part (diabetic nephropathies, malignant nephrosclerosis, infections, and intoxications). As has been pointed out in the foregoing discussion, this is certainly also true of vasculitis and of granulomatosis. One cannot, on the basis of the morphologic appearances alone, draw definite conclusions as to the pathogenesis of any one of these types of tissue change; identity of appearance does not imply similarity of etiology.†

Admitting that any one of the characteristic lesions of Wegener's granulomatosis may conceivably result from causes other than allergic, each of them has also been clearly associated with hypersensitive states, and the concurrence in the same syndrome of at least three such manifestations (necrosis and granuloma; arteritis; glomerulitis, trabeculitis, and folliculitis) renders the inference almost ineluctable that we are here dealing with a disease of hypersensitivity. In this almost all authors who have recognized the syndrome agree. It has been most frequently assumed, when the type of allergy was named, that a process in the nature of an Arthus phenomenon was involved.‡ However, we have no clinical immunological information to permit more than a surmise regarding the kind of allergy (bacterial or Arthus type) involved.

To explain the peculiar concentration of lesions in the respiratory tract, it might be considered that (*a*) this site is the primary locus of attack of a noxious agent, probably microbial, which is present locally in highest concentration for the longest duration and/or (*b*) that the respiratory tract tissues are most highly sensitized, that is, they form the first, most susceptible, shock tissues. Generalized vasculitis, nephritis, and disseminated granulomata probably occur only later in the course of the disease, perhaps dependent either upon the development of a state of generalized vascular hypersensitivity or upon sudden or intermittent free access to the circulation of allergenic substance from the primary respiratory site (whether the original incitant or some product of the injured shock tissues) or upon both.

NOSOLOGY

In attempting to assign some classification to this syndrome of necrotizing respiratory granuloma, angiitis, and nephritis, one is confronted by its close relationships to other described subgroups of arteritis, on the one hand, and to pure

References 64 through 66.
* References 28 through 30.
† References 67 and 68.
‡ References 16 and 32.

necrotizing or granulomatous processes, on the other. Most writers, like Wegener,§ have given it place as a particular species of periarteritis, but other experienced pathologists (Aschoff, Fahr, Schurrmann,[2] Mallory,[13] Johnsson [11]) have thought it a disease *sui generis*.

From the pathogenetic point of view, a precise species differentiation is not very important, perhaps, when one considers a continuous spectrum of tissue changes from pure necroses and granuloma formation to pure angiitis, with intermediate combinations, all of them manifestations of a state called "rheumatic" || or "allergic." ¶

But in our present state of knowledge, from the standpoint of etiology and diagnosis, it is of great moment to define carefully such separable syndromes, for only in this way can recognition be assured and those investigations be undertaken which will add to our knowledge of the causation of this disease.

SUMMARY

The pathology of 7 cases of a syndrome (Wegener's granulomatosis) presenting severe destructive lesions of the respiratory tract, arteritis, and nephritis is described, and the findings in 22 other reported cases are tabulated. This disease is characterized by aggressive necrotizing granulomata of the respiratory tract, generalized angiitis, necrotizing glomerulitis, and, frequently, disseminated granulomata. The anatomic findings are discussed, and those features of Wegener's granulomatosis differentiating it from the similar and related disease patterns of necrotizing granulomatous processes, generalized arteritides, and mixed allergic angiitis and granulomatosis are presented. Reasons, based on pathological findings, for considering Wegener's granulomatosis a disease of hypersensitivity are offered.

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ATYPICAL ADENOMA OF THE THYROID

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CERTAIN adenomas of the thyroid are occasionally encountered which, because of their unusual appearance, cause doubt as to their true nature. The atypical character of these tumors is evidenced grossly by their extraordinary solid, fleshy appearance. Microscopically, they show compactness of cell arrangement and bizarre distribution of epithelial and stromal elements. Graham [1] recognized these lesions and encountered difficulty in distinguishing them from tumors of malignant type. He found it necessary to rely on one single feature, vascular invasion, as an indication of malignancy. In his series of 47 cases, 16 patients were traced for a period exceeding five years, and in each there was a benign course.

So far as can be determined, these tumors have found recognition only in the one publication, and their gross and histologic features have not been fully explored. Because these atypical adenomas of the thyroid are readily confused with neoplasms of malignant type, a study of them as a group was considered advisable.

MATERIALS AND METHODS

The study was limited to those thyroids, obtained at surgery, which contained an encapsulated nonpapillary nodule. These lesions were usually solitary, or they were distinctive because of size or unusual appearance. In all, 2,452 glands were examined. Sections were routinely stained with hematoxylin and eosin and, in addition, the Wilder technique for reticulin was used, followed by Van Gieson counterstain. A search for mitoses was made with the oil immersion lens over a calculated area of 50 sq. mm. in the most cellular portion of the tumor.

PATHOLOGY

The largest number of the encapsulated lesions reviewed were simple adenomas characterized by well-differentiated follicles of variable sizes. The remainder were those of fetal, embryonal, and atypical patterns. Only those tumors with small follicles nearly or completely closed were included in the fetal group; those with tubular or cordlike patterns were regarded as embryonal. The adenomas formed by oxyphilic epithelium, regardless of the type of follicular structure, were tabulated separately. The cells of the rare oxyphilic tumor were large and of so-called

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Hürthle cell type. The atypical group, comprised of 57 tumors, representing 2.3% of the specimens examined, are defined below in detail. Distribution of types is shown in Table 1.

GROSS FEATURES

The atypical adenomas ranged from a diameter of 1 to 12.5 cm., but the majority were large, and the average was 5.7 cm. Each was completely defined by a distinct capsule. They were most commonly formed by opaque, gray-white tissue, although yellow-tan, brown, and red-brown colorings were observed. All lacked the gelatinous or semitranslucent character of the usual adenoma. The appearance of the tumor is illustrated in Figure 1. Though the adenoma with intact capsule was firm to hard, after sectioning the tissue was commonly friable, at times soft. Thirteen (23%) were cystic (Fig. 2). Ten (18%) appeared in multinodular goiters.

MICROSCOPIC FEATURES

With the routine hematoxylin and eosin stain, there were three architectural variants: (1) closely packed follicles, often nonluminated, (2) solid columns, often

TABLE 1.—*Classification of 2,452 Adenomas*

Simple	2,083
Fetal	215
Oxyphilic	80
Atypical	57
Embryonal	17
Total	2,452

with little intervening stroma (Figs. 3 and 4), and (3) solid sheetlike or diffusely cellular masses (Fig. 5). These patterns most frequently formed the predominant or entire structure of the adenoma, but infrequently they occurred as focal areas in an otherwise differentiated adenoma. Often two or all three of them were present in a single lesion.

Stroma.—The absence of a pale-staining, edematous stroma was a prominent feature, but fibrous trabeculae occasionally were seen. Capillary networks separated epithelial groups of variable sizes. Some tumors, appearing to be of solid structure with the routine stain, revealed fine, argentophilic and red-stained fibers outlining small, closed follicles and cell cords when the reticulin and Van Gieson method was employed.

Capsules.—Capsules were distinct, were not invaded, and were varied in thickness.

Necrosis.—Necrosis was noted in only three tumors (5%).

Cytology.—The majority of the tumors were formed by cells of small or medium size with dark-staining, round nuclei. There was some variation in nuclear size. Nucleoli usually were not prominent. Giant nuclear forms, chiefly associated with oxyphilia, were found in 5 (9%) of the 57 tumors. Six adenomas (10%) were formed by oxyphilic epithelium and were selected because of unusual solidness of structure. The rare cytologic types were as follows: one spindle cell (Fig. 6), one clear and granular cells, two focal clear cell, and two focal squamous cell (Fig. 7).

Mitoses.—Mitoses were present in 20 (35%) of the 57 tumors: three-quarters of the 20 showed less than 5 mitoses per 50 sq. mm., and the remainder, 6 to 10 mitoses. No atypical mitoses were seen.

Degree of Atypia.—Atypia was separated into three categories: (1) slight, (2) moderate, (3) marked. The categories are based on the architectural patterns as

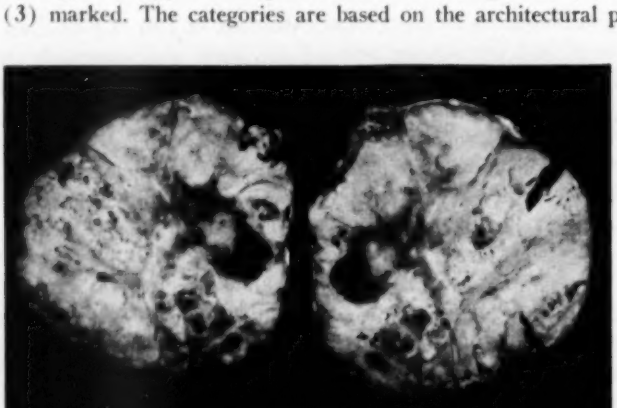

Fig. 1.—Adenoma formed by opaque, gray-white tissue. Darker areas are zones of hemorrhage.

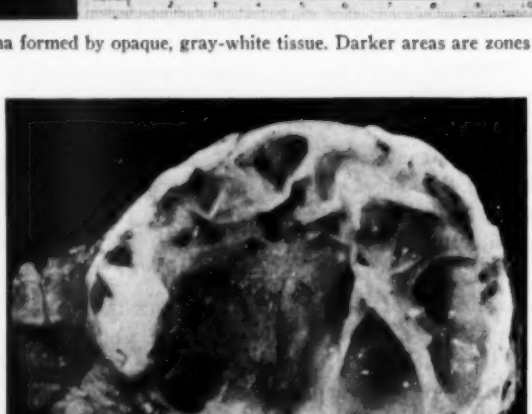

Fig. 2.—Cyst formation in adenoma otherwise formed by gray and white tissue. Zones of fibrosis were more extensive than usually encountered.

seen in the routine hematoxylin and eosin preparations supplemented by the reticulin and Van Gieson stains. The tumors that were regarded as showing slight atypia revealed a structure of uniform cords and small follicles, as seen in fetal and embryonal adenomas. The cells, however, were unusually closely packed, and the edematous stroma was entirely lacking (Figs. 3 and 4). Atypia was regarded of

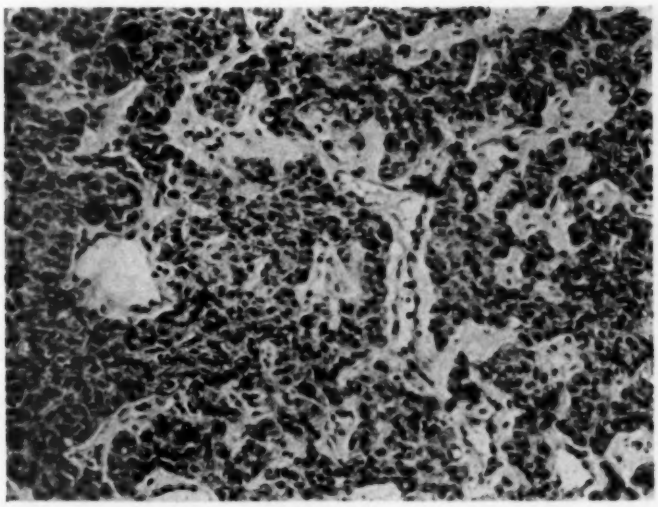

Fig. 3.—Adenoma formed by anastomosing, irregularly oriented, cellular cords of variable width. Intervening stroma is fibrous. Hematoxylin and eosin; × 120.

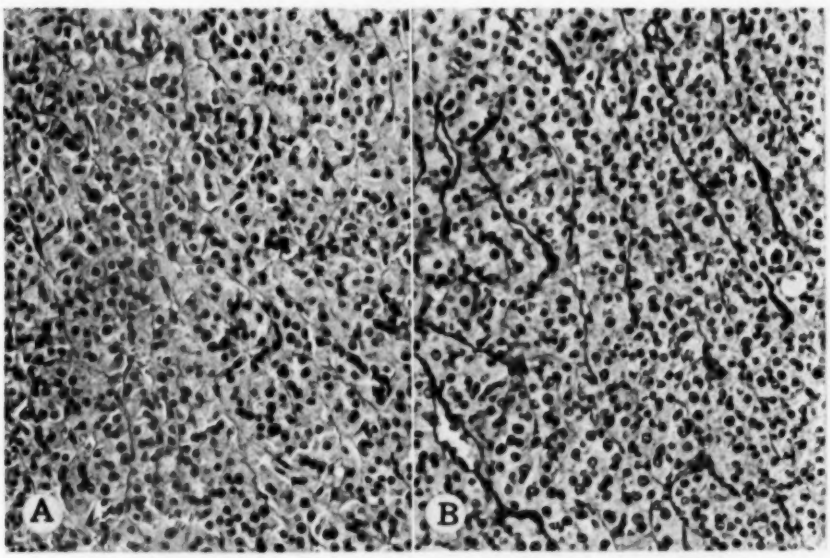

Fig. 4.—*A*, tumor formed by compactly arranged, irregular, solid columns of cells indefinitely separated by capillaries and faintly outlined fibers. Hematoxylin and eosin; × 190. *B*, microscopic field similar to *A* with fine stromal elements definitively outlined. An edematous stroma was entirely absent. Reticulin and Van Gieson; × 190.

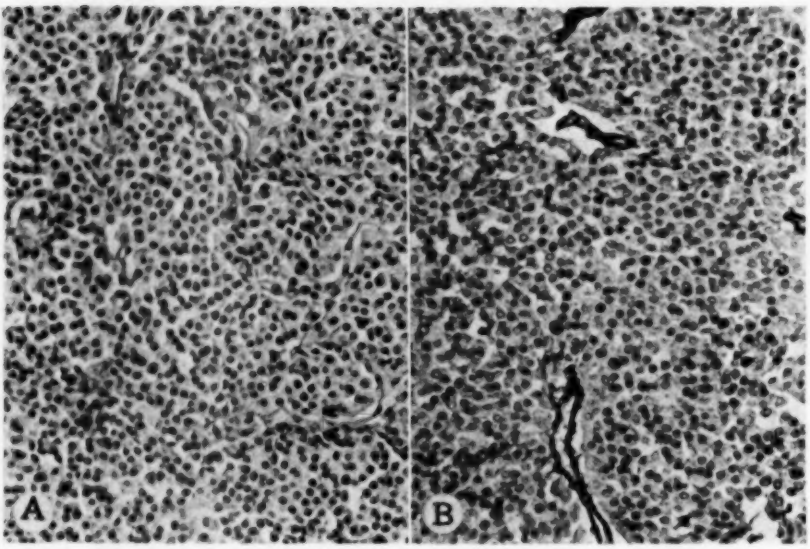

Fig. 5.—*A*, solid sheetlike arrangement of cells and ill-defined small vessels. Hematoxylin and eosin; × 190. *B*, similar microscopic field showing mass of epithelial cells interrupted only by isolated vessels and their accompanying stroma. Reticulin and Van Gieson; × 190.

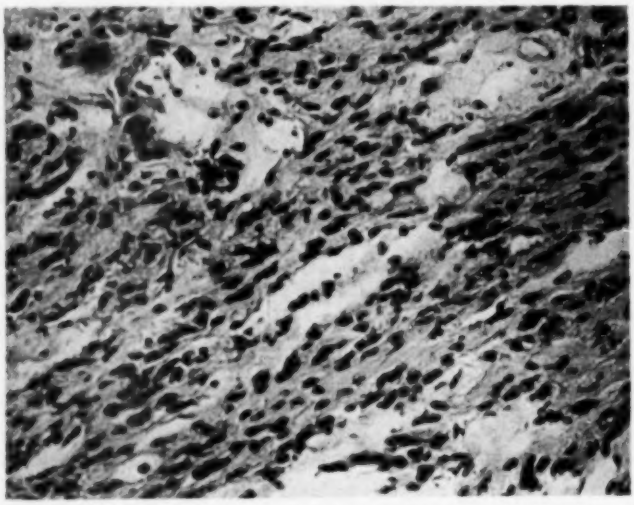

Fig. 6.—Elongate cells forming tissue not identifiable as thyroid. Rare follicles were observed elsewhere. Hematoxylin and eosin; × 180.

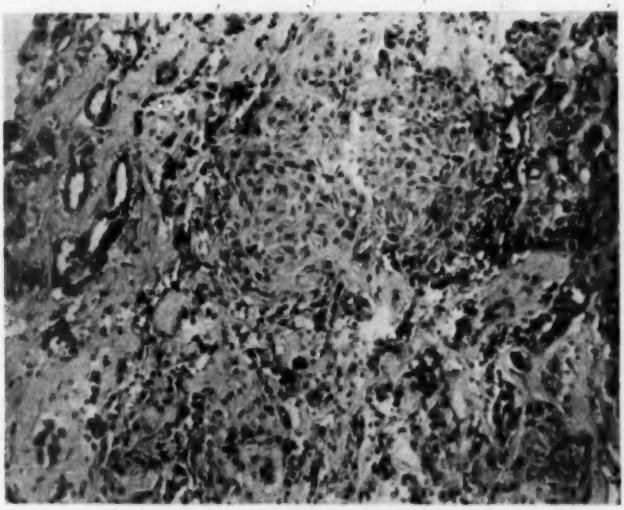

Fig. 7.—Portion of adenoma showing foci of nonkeratinized squamous cells in fibrous stroma. Hematoxylin and eosin; × 100.

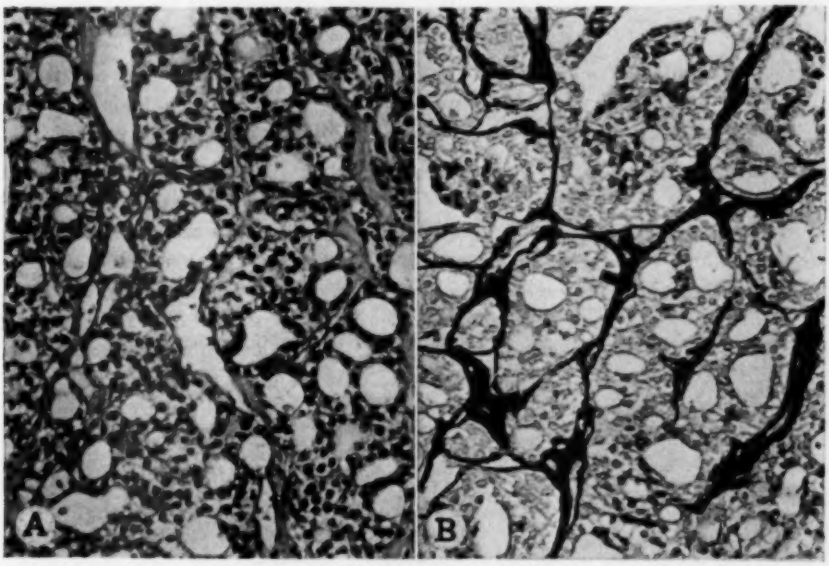

Fig. 8.—*A*, closely packed follicles of variable size arranged in groups, separated by irregular short, fibrous trabeculae. Hematoxylin and eosin; × 190. *B*, area in same tumor demonstrating reduplication of lumens. Epithelial groups separated by dense fibrous trabeculae which were definitely stained. This histologic pattern is similar to that of adenocarcinoma. Reticulin and Van Gieson; × 190.

moderate grade in those lesions with closely packed cells in broad, solid sheets (Fig. 5), in part or wholly without follicular outlines and interrupted only by irregular fibrous trabeculae. A third group with marked atypia was characterized by reduplication of lumens irregularly spaced in sheets or broad columns of cells unbroken by reticulin (Fig. 8). In this third group, there were varied sizes and orientations of the cellular masses. There was no significant difference in the numbers of mitoses present in the three categories. The incidence of the three grades is shown in Table 2.

Comparison with Fetal and Embryonal Adenomas and with Angioinvasive Encapsulated Tumors.—A group of 20 encapsulated nonpapillary tumors with angioinvasion and a series of 20 fetal and embryonal adenomas were studied for comparison with 20 consecutive atypical adenomas. Mitoses were found in 8 of the adenomas and 11 of the angioinvasive neoplasms as compared with 9 of the sample group of atypical adenomas. The number per unit area (50 sq. mm.) was lowest in the fetal and embryonal adenomas (maximum 4, mean 1), and approximately equal in atypical adenomas (maximum 10, mean 2) and in the angioinvasive tumors (maximum 11, mean 2). An architectural comparison, Table 2, reveals that the majority of the atypical group showed slight or moderate atypia, whereas, in the encapsulated angioinvasive tumors the grade was moderate or marked.

TABLE 2.—*Atypia in Atypical and Encapsulated Angioinvasive Tumors*

	Slight	Moderate	Marked
Atypical adenomas	22	28	7
Encapsulated angioinvasive tumors	2	8	11

CLINICAL FEATURES

The lesions were found with greater frequency in women than in men: there were 43 women and 14 men, giving a ratio of three to one. The ages of the patients at the time of operation ranged from 18 to 65 years (an arithmetic mean of 38.7 years). A study of the histories indicates that these tumors had been present clinically for periods ranging from 2 months to 25 years (an arithmetic mean of 8.9 years). Thus, the ages at onset of appearance of the lesions were from 11 to 56 years (an arithmetic mean of 30.7 years). No patient showed clinical features of hyperthyroidism. Treatment was by lobectomy, by subtotal lobectomy, or by subtotal thyroidectomy. Postoperative irradiation was administered in only a few of the cases, and these were early in the series.

PROGNOSIS

Thirty-one cases were traced for periods of 5 years or more after operation, 13 of these for over 10 years, and 4 for over 20 years. None of these patients has shown recurrent neoplasm. Of the remaining 26 patients, 12 were operated upon within the last five years; 1 died postoperatively, and 13 could not be contacted, the majority having come early in the series.

COMMENT

Interest in the possibility of metastasis of encapsulated tumor of the thyroid was aroused as early as the year 1876, by Cohnheim's [2] report of a case in which lung and bone metastases occurred and yet the thyroid contained only two noninfiltrating nodules. Although vascular invasion was described in the report of the thyroid tumor, Cohnheim did not realize its true significance. Other reports of a so-called benign metastasizing goiter followed, and these were well summarized by Simpson [3] in 1926. He found microscopic examination of the thyroid had been made in only 29 of 77 cases described and concluded that the entity of benign metastasizing goiter did not exist. Graham [1] in 1924 established the existence of adenomas that have the histologic architecture of carcinoma but have benign behavior. On studying 108 epithelial tumors originally diagnosed as carcinomas, he found that 43 had a malignant histologic appearance but were noninvasive. Of these, he traced the histories of 16 and found no instances of recurrence or metastasis. He emphasized the importance of invasiveness and stated that encapsulated tumors without evidence of blood vessel invasion were benign regardless of their microscopic appearance.

Warren [4] recognized the significance of vascular invasion in the diagnosis of malignancy in encapsulated tumors. He found no instance of malignant behavior in a group of 1,080 adenomas without vascular invasion. However, of 34 patients with angioinvasive encapsulated tumors, two died with multiple metastases. In 1947 he [5] reiterated his views concerning the benign course of the noninvasive group of adenomas and stated that he had seen only one case in which there were subsequent metastases without demonstrable vascular invasion.

Habermel [6] recognized autonomous new growth in an adenoma, stating that it was demonstrated by increased cellularity, granular pale-staining epithelium, hyperchromatism, abundant mitoses, growth in solid sheets or long columns, and scanty stroma. He stated that malignancy does not exist without local or systemic invasion, and that blood vessels containing tumor cells are often found before the capsule is broken.

Zimmerman, Wagner, Perlmutter, and Amromin [7] classified with the carcinomas those borderline encapsulated tumors showing proliferation, mitoses, and cellular pleomorphism, without vascular or capsular invasion. They included these under the term "malignant adenoma," and applied this designation to tumors in which a positive diagnosis cannot be made.

Thus, there has been some published recognition of this group of neoplasms but, in general, only two categories of encapsulated tumor are recognized: adenoma and the so-called angioinvasive adenoma. The atypical adenomas differ from the usual types in one or more features: solid architecture, disorderly arrangement of follicular and cord-like components, irregular fibrous trabeculation, reduplication of follicular lumens, and absence of edematous stroma. The increased cellularity of the angioinvasive group, as compared with the ordinary follicular adenoma, was recognized by Warren,[4] who classified the architecture of 34 such tumors as embryonal or fetal, with one exception that was unclassified.

We have noted not only cellularity, but also atypia as constant features of angioinvasive adenomas. In fact, those atypical adenomas with moderate or marked atypia were histologically indistinguishable from the great majority of encapsulated angioinvasive tumors. Because of this similarity, classification is most difficult in

the more atypical adenomas. The need for accuracy in making the classification is emphasized by the great difference in prognoses of these two types of lesion. In all of the patients with atypical adenomas followed five years or longer in this series, the clinical course was benign. In contrast, there was frequent recurrent or metastatic disease in 25 patients with encapsulated angioinvasive tumors that the authors [8] have been able to trace adequately. Three-fourths of such malignant manifestations occurred within five years, a fact that would seem to emphasize the adequacy of the follow-up period of this group of atypical adenomas. Benign behavior was characteristic also of the 16 non-vessel-invading neoplasms reported by Graham [1] and those reported by Warren,[5] with the exception of one. The three reported by Zimmerman and co-workers [7] cannot be evaluated as to prognosis.

An important feature in the diagnosis of encapsulated tumors of the thyroid is adequate evaluation of the capsular area. Graham [1] stated that innumerable blocks were not necessary, but he made no specific recommendation. In our review of a comparative series of angioinvasive tumors, involved vessels were found frequently in each of several blocks. There were a few tumors, however, in which invaded vessels were sparse and multiple blocks—in one instance seven blocks—were necessary to demonstrate their presence. It is advisable to examine at least four blocks that include the capsule in each cellular adenoma. When these fail to reveal vascular invasion, it has been helpful to examine a second series of four blocks. In instances of marked atypia, this study has been repeated with four additional blocks. In general, however, if no angioinvasion or capsular penetration is present in eight blocks of adequate size, invasiveness is not a feature of the neoplasm.

The presence of mitoses is not diagnostic of malignancy in an adenoma, as shown in the adenomas of fetal and embryonal types. The frequency per unit area, however, was less in these than either in atypical adenoma or in the angioinvasive tumor.

Giant nuclear forms were infrequent. When found in an encapsulated neoplasm, they are not regarded as evidence for the diagnosis of malignancy. In this series they were mostly associated with oxyphilia and were regarded as involutional forms. Tumor giant cells that are of significance are found in nonencapsulated tumors and then in association with growth activity evidenced by frequent mitoses and cellular dedifferentiation.

Among the types of infrequent occurrence were those atypical adenomas with clear cells. In two instances these occurred as focal groups, and in one instance the entire tumor was of clear and granular cell structure. The presence of nodules formed by such cells always necessitates consideration of the possibility of metastases from a renal tumor. The adenoma comprised of clear and granular cells was from a patient still surviving without evidence of tumor seven years after her operation for thyroid disease.

Is the atypical adenoma in reality an encapsulated adenocarcinoma without apparent vascular invasion? On the basis of histologic comparison with known vessel-invading neoplasms, preinvasive malignancy is indeed a possibility, especially in those of markedly atypical character. Furthermore, the mere fact that the tumor has been removed with no subsequent recurrence does not disprove such an assumption. Nevertheless, because of obvious importance to the patient, until

there is further clinical evidence of malignant behavior, or until such time as a positive criterion of cancer substantiates such a possibility, these tumors should be classified as atypical adenomas.

SUMMARY

Fifty-seven (2.3%) of 2,452 thyroid adenomas were of atypical gross and microscopic appearances.

No evidence of recurrences was found in 31 patients followed five years or longer after local surgical removal.

Although the majority of the atypical adenomas grossly and histologically resembled encapsulated angioinvasive tumors, no vascular invasion was apparent.

Adequate study of the capsular area of these tumors through examination of multiple blocks is essential to exclude or confirm angioinvasion or early capsular penetration. In the absence of these recognized morphologic signs of malignancy, an encapsulated nonpapillary tumor of the thyroid with an unusual histologic pattern should be classified as an atypical adenoma.

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BILATERAL OXYPHILIC GRANULAR CELL ADENOMA OF PAROTID

Report of a Case

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THE OCCURRENCE of oxyphilic granular cell adenomas of the salivary glands, while infrequent, is by no means rare.* The studies of apparently normal salivary glands by Hemperl, Steinhardt, and Meza-Chávez [7] indicate that the "oncocytes" occur at multiple sites in the smaller ducts and that they increase in numbers with age; furthermore, Gruenfeld and Jorstad,[4] Harris,[5] and Meza-Chávez [7] believe that oxyphilic granular cell adenomas are formed by a coalescence of multiple nodules or have a multicentric origin from ductal epithelium.

A perusal of the available literature fails to reveal a report of the bilateral occurrence of this type of tumor. Perhaps this is due to the relatively few cases of this type of adenoma reported. The case to be reported was bilateral and microscopically appeared to be of multicentric origin on each side.

F. F., a 65-year-old white woman, stated that she had had a swelling of both cheeks for many years. After the extraction of a right lower molar in 1920, the lower right cheek increased in size slowly and painlessly over the following four years. Incision and drainage of the cheek was done without relief, slight weakness of the right corner of the mouth resulting. In 1942, the left lower third molar was extracted, following which the left cheek began to swell progressively in the same manner as had the right cheek, and considerable pus drained into the patient's mouth every day. Penicillin, given from time to time, lessened the enlargement.

The significant findings on examination were the considerably enlarged parotid glands, the right being the larger. The tumor masses, which gave the patient the appearance of a "squirrel with nuts in his cheek," were multinodular, fairly soft and nonfluctuant, tender on palpation, and not fixed to the mandible. Each mass extended 1 cm. posterior to the ear lobule, extending up to the zygoma, downward 3 cm. below the ear lobule, and forward to the midcheek. There was a small, white scar, 2 cm. long, just beneath the right ear lobe. Clear fluid could be expressed from Stensen's ducts, which were not particularly pronounced at their papillae.

Laboratory examination, including urinalysis, complete blood count, Wassermann and Kahn tests, nonprotein nitrogen, creatinine, blood sugar, and blood chloride showed values within normal limits. Sedimentation rate was 12 mm. in an hour. Electrocardiogram showed normal tracing. Sialadenogram with methiodal (Skiodan) sodium showed apparently normal ramification of the duct system throughout both parotids, but no obstructions were noted.

On May 29, 1951, with the patient under endotracheal anesthesia, both sides were explored. Multilobular, soft, brown, friable masses were encountered bilaterally as the capsules of the tumors were exposed. On the left, even though the encapsulated mass, not being adherent to

From the Departments of Surgery (Dr. Robinson) and Pathology (Dr. Boley) of the University of Kansas Medical Center.

* References 1-8.

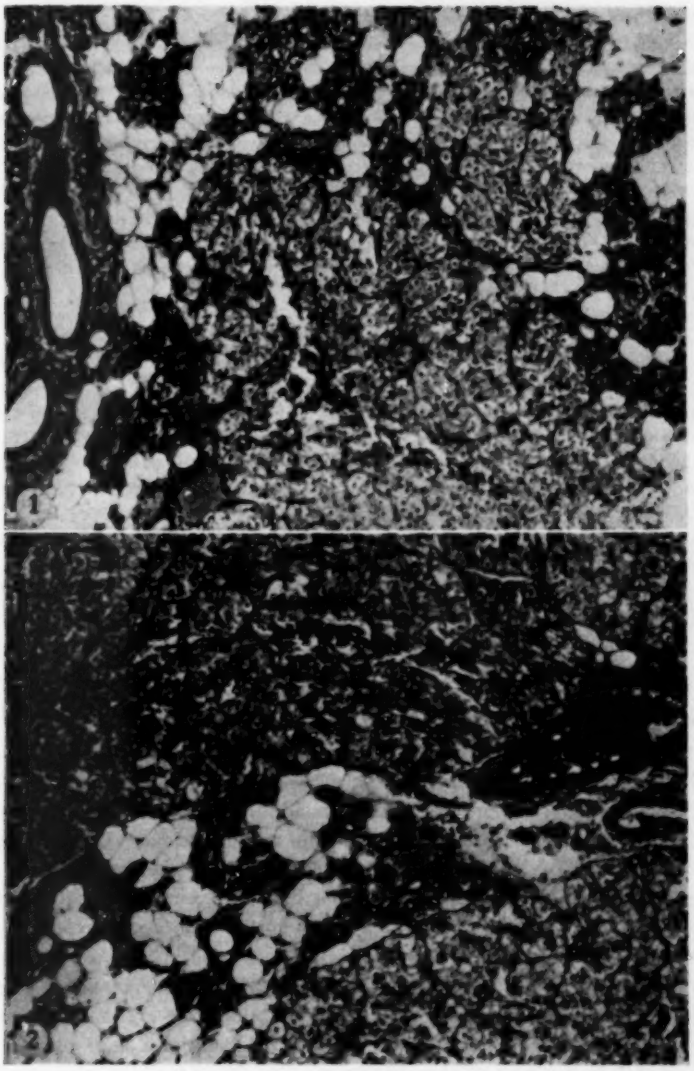

Fig. 1.—Section of parotid which reveals the cells of the normal acini to be dark while the lighter oxyphilic granular cells are in nests with no apparent capsule. Hematoxylin and eosin stain; × 100.

Fig. 2.—Section through encapsulated tumor showing parotid duct and fatty parotid gland tissue apparently included by tumor. Hematoxylin and eosin stain; × 100.

the surrounding tissue, was easily shelled out, there was no distinct visible line between the tumor and the grossly normal-appearing parotid. The facial nerve was located and not disturbed. The parotid beneath the tumor had the usual multilobular surface.

On the right side, owing to the previous incision, the tumor was slightly adherent to the subcutaneous fascia and more difficult to remove. Although the main trunk of the facial nerve was found and most of the tumor mass removed, a small portion of tumor was left undisturbed because it so intricately involved the region about the facial nerve, probably because of scar. Since this was surely a benign tumor, with its course over 31 years, radical resection with removal of the seventh nerve seemed unwise, and a somewhat piecemeal removal was considered acceptable.

Regular follow-up examinations every four months have revealed slight increase in size of the remaining tumor on the right but no subjective complaints. The facial nerve weakness on the right remains unchanged. There has been no recurrence noted in the left parotid.

PATHOLOGIC DESCRIPTION

Gross Examination

The specimen consisted of several fragments of partially fixed tissue from the right parotid gland and weighed 28 gm. The many irregularly shaped fragments, measuring up to 3.5 cm. in greatest diameter, were grey and had the lobular appearance of salivary gland. Three fragments presented more rounded, coarsely lobulated surfaces, and each measured 1.5 cm. in diameter. These were soft to rubbery in consistency and on cut section had a yellowish-tan, homogeneous, cellular appearance.

The specimen from the left parotid weighed 36 gm. and was in six major pieces, with numerous additional small fragments. The largest piece measured 4.5 by 3.5 by 2 cm., the smallest 2 by 1.5 by 1 cm., and all had rounded, coarsely lobulated surfaces. The cut section revealed a soft, crumbly, cellular, yellowish-tan tissue with grey mottling. Very little apparently normal parotid tissue was present.

Microscopic Examination

The tumor nodules from the two sides were identical microscopically. In most areas the tumor was well encapsulated, fragments of normal parotid tissue occasionally being attached to the capsular surface. In some areas (Fig. 1), the capsule was less evident and tumor was seen to invade the fatty parotid tissue. In other fields, parotid acini and ducts were seen within the encapsulated areas of the tumor (Fig. 2). The tumor had an alveolar pattern formed by delicate fibrous strands bearing thin-walled blood vessels. The tumor cells varied in pattern from oval nests to elongated cords which resembled adrenal cortex; however, occasionally acini were seen.

The cells were large, polyhedral to rounded, with abundant pale to acidophilic cytoplasm. The more acidophilic cells contained acidophilic granules, the pale cells a lacy cytoplasm. The nuclei were centrally placed, uniform, and round to oval, and showed fine stippling of the chromatin. The nuclei in the tumor were larger and less chromatic than seen in the adjacent parotid acini. The paler staining areas did not contain mucin or fat. In some areas a gradual transition from parotid acini to tumor was noted as evidenced by (1) increasing size of cells; (2) change in character of cytoplasm, from basophilic to acidophilic; (3) increase in size and decrease in staining of the nuclei; (4) shift of the nuclei from a basal to a more central location in the cell. Occasional parotid ducts were dilated and contained acidophilic amorphous material.

COMMENT

Foote and Frazell[9] stress the infrequence of bilateral tumors of the salivary glands, none being present in their series of 877 tumors of major salivary glands, and list four cases in the literature. In the series of 103 salivary gland tumors at the University of Kansas Medical Center, three persons with bilateral tumors have been encountered, one being the case reported, another a case with bilateral papillary cystadenoma lymphomatosum, and the third a case with bilateral mixed tumors, one of which was partially calcified. Owing to the infrequency of bilateral tumors of the parotid glands, especially oxyphilic granular cell tumors, it seems worth while to report this case.

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PRIMARY PULMONARY ARTERIAL DISEASE

Observations with Special Reference to Medial Thickening of Small Arteries and Arterioles

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THE EXISTENCE of primary pulmonary arterial disease as an entity has been the subject of discussion for many years.* As investigation has proceeded, many causes for secondary pulmonary arterial disease have been discovered,† and consequently primary arterial disease has been questioned. In order to evaluate the concepts of this disease all cases in the files of the Massachusetts General Hospital so diagnosed have been reviewed and have been compared with a large number of lungs showing secondary pulmonary arterial disease. From the last 10,000 autopsies performed at the Massachusetts General Hospital, only two cases fulfill the criteria for the primary form.

REPORT OF CASES

CASE 1.‡—A 6-month-old white boy was admitted to the Massachusetts General Hospital because of vomiting and cough of three weeks' duration. Pregnancy and delivery were uneventful. Development was normal; there was no cyanosis. Three weeks before entry he began to cough. There was frequent post-tussive vomiting. The vomiting became severer and the patient pale and weak. Several blood transfusions were given, raising the hemoglobin from 40 to 110%. A roentgenogram of the chest was negative. There was slight general improvement. Eight days prior to admission the mother noted that the child's fingers and toes were blue. His temperature was normal. On the day before entry he became acutely ill with respiratory difficulty and cyanosis.

Two siblings, aged 2 and 4 years, were normal.

On physical examination the baby was pale and fretful, with cyanosis of the lips and nail beds. The throat was congested and filled with mucus. The heart was normal. The lungs were clear anteriorly, but showers of rales were heard in both upper lobes posteriorly, and these areas were flat to percussion. The liver edge was felt 3 cm. below the right costal margin. Neurological examination was negative.

The temperature was 102 F., pulse rate 160, and respirations 60 to 80 per minute.

Examination of the blood revealed a red cell count of 4,000,000 with a hemoglobin content of 87%. The white cell count was 51,000, with 55% neutrophiles, but on the following day it was 18,400, with 79% neutrophiles. In the blood smear there was marked anisocytosis and many normoblasts were present. No sickling of the red cells occurred. In the osmotic fragility test hemolysis began at 0.45% and was complete at 25%. The CO_2-combining power was 15.7 mEq.

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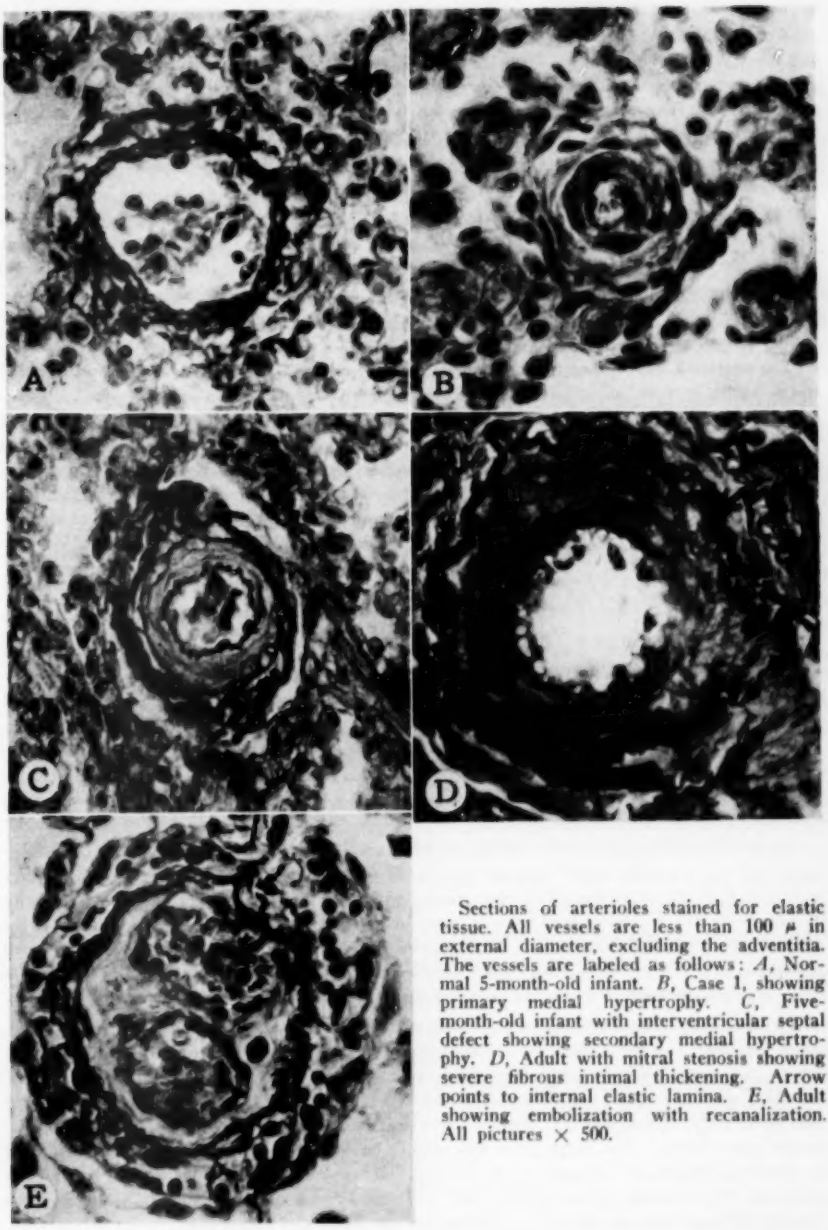
* References 1 and 2.

† References 3-10.

‡ This case was previously reported in the case records of the Massachusetts General Hospital.[13]



Sections of arterioles stained for elastic tissue. All vessels are less than 100 μ in external diameter, excluding the adventitia. The vessels are labeled as follows: *A*, Normal 5-month-old infant. *B*, Case 1, showing primary medial hypertrophy. *C*, Five-month-old infant with interventricular septal defect showing secondary medial hypertrophy. *D*, Adult with mitral stenosis showing severe fibrous intimal thickening. Arrow points to internal elastic lamina. *E*, Adult showing embolization with recanalization. All pictures × 500.

per liter. The nonprotein nitrogen was normal. Throat and stool cultures were negative for pathogens. A tuberculin test (1:1,000) was negative. The blood Hinton test was negative.

Roentgenograms of the bones showed no evidence of disease. The chest was unusually large, and the diaphragms were low. The size and shape of the heart were normal, but the mediastimum appeared shifted to the left, although there was no change with respiration. The pulmonary markings were increased bilaterally. Repeated electrocardiograms showed right ventricular strain, suggesting a congenital lesion with acute right ventricular failure.

In the hospital the patient was maintained in an oxygen tent but became cyanotic when removed.

On the eighth hospital day the red cell count was 5,100,000, with a hemoglobin content of 100% and a white cell count of 11,200. He gained weight, and a marked pitting edema was noted by the 11th hospital day. Clubbing of the fingers developed, and the liver became enlarged until the edge was felt at the iliac crest. Three weeks after entry, after his usual bath he became quite cyanotic and remained so after being returned to the oxygen tent. He died quietly after 45 minutes.

The necropsy was limited to examination of the heart and lungs. The spleen was palpated and found to be firm and enlarged. The liver appeared normal. The lungs weighed 125 gm. (normal, 90 gm.), and although they appeared reasonably well distended, they were subcrepitant and doughy. The cut surfaces were dry. The heart weighed 55 gm. (normal, 34 gm.), and there was marked hypertrophy and slight dilatation of the right ventricle, which measured 0.5 cm. in thickness (normal, 0.2 cm.). The left ventricle was 0.6 cm. thick. The right atrium was distended. The valves were negative. The pulmonary artery measured 3.5 cm. in circumference just above the valve cusps and appeared normal throughout its branches.

Microscopically the heart was normal. In the lungs there was pronounced medial thickening of all the small arteries and arterioles, particularly in those measuring less than 100 μ in diameter. This thickening was so marked that the media of these arteries was equal in thickness to the media of comparable systemic vessels. Throughout the lung there was marked atelectasis with moderate distention of the alveolar ducts. Some of the alveolar walls were moderately edematous, and many of the alveolar spaces contained pigment-filled macrophages.

Case 2.§—A 10-year-old boy was admitted to the Massachusetts General Hospital because of severe congestive heart failure.

He had always been in good health and had developed normally until two years before entry, when his parents noted that he tired easily and became blue and short of breath after exertion. A diagnosis of congenital heart disease was made and his activities restricted, but with no improvement. A few months later he vomited two "mouthfuls" of bright red blood. Eight months before admission his legs became swollen and orthopnea developed. He was restricted to bed for a few days, with considerable improvement, but the symptoms of congestive failure soon returned and increased progressively, with the edema gradually spreading to his back.

Physical examination revealed a well-developed but undersized and dyspneic boy with cyanosis of the lips. The chest was barrel-shaped, with bulging of the precordium and the sternum, and the ribs were tender to percussion. Fine crepitant rales were present over both lung bases, and there were absence of breath sounds, dullness, and diminished tactile fremitus over the right base posteriorly. Breathing was labored and chest expansion poor. The neck veins were distended and pulsating. The radial pulse was of poor quality, and no blood pressure reading could be obtained. The heart was enlarged to the left axillary line, and the right cardiac border was percussed 5 cm. outside the midsternal line. The sounds were of fair quality. The rhythm was regular, but a presystolic gallop could be heard over the entire precordium except at the apex.

§ This case has previously been reported in the case records of the Massachusetts General Hospital.[14]

The pulmonic second sound was louder than the aortic but not markedly accentuated. There were no thrills or murmurs. The abdomen was protuberant, and a definite fluid wave could be elicited. The liver edge was 3 fingerbreadths below the right costal margin and slightly tender. There was marked edema of the legs and sacrum.

The temperature was 99.5 F., the pulse rate 90, and the respirations 27. The urine was negative. The red cell count was 4,650,000, with a hemoglobin level of 75%. The white cell count was 9,150, with 88% neutrophiles. An electrocardiogram showed right axis deviation.

The child was treated with digalen, mercurial diuretics, and morphine but responded very slowly. The edema spread to the penis and scrotum.

On the fourth hospital day, a portable roentgenogram of the chest showed the heart to be grossly enlarged, particularly to the right. It had the shape of a water bottle; the margins were smooth and sharp and the usual chamber outlines were not seen. The left main bronchus was slightly elevated, and the angle of the carina was increased. The outline of the diaphragm at both bases was obliterated by an increased density. The exact position of the aorta was not established. In spite of treatment the edema failed to clear and the patient gradually became more dyspneic and cyanotic; use of an oxygen tent became necessary. On the seventh hospital day the presystolic gallop was replaced by a reduplicated second sound. The boy died on the following day.

Necropsy was limited to examination of the heart and lungs, and samples of the spleen, liver, and kidney were permitted. Each pleural cavity contained 200 cc. of clear brownish fluid. The lungs together weighed 700 gm. (normal, 250 gm.). Crepitus was diminished, particularly in the lower lobes, which were very firm, with reddish-gray, homogeneous, dry, cut surfaces. In the left lower lobe was a V-shaped infarct 1 cm. in diameter. The pericardium contained about 100 cc. of clear straw-colored fluid. The heart weighed 200 gm. (normal, 116 gm.). The water-bottle shape seen by x-ray was due to tremendous dilatation of the right atrium, which before removal of the heart made up a much larger proportion of the presenting surface than normal. The right ventricle was also dilated and hypertrophied, measuring 0.5 cm. in thickness (normal, 0.3 cm.). The left atrium was moderately dilated, and its endocardium was diffusely and markedly thickened and whitish and contained a few small foci of calcification. The left ventricle was normal, measuring 1.3 cm. in thickness. The valves were negative. The entire heart was rotated 30 to 40 degrees in a counterclockwise direction, so that its greatest diameter was transverse rather than vertical. The pulmonary artery showed a very slight degree of atherosclerosis.

Microscopically the heart was normal except for the endocardium of the left atrium, which showed marked diffuse and uniform fibrous tissue thickening with a few flecks of calcium embedded in it. The muscle fibers showed no abnormal amount of glycogen. There was no evidence of rheumatic infection. In the lung, the small arteries and arterioles showed medial thickening which was only slightly less marked than in Case 1. In addition, there was slight fibrous intimal thickening. A marked chronic bronchitis was present, and there were a few areas of focal scarring. The alveoli were edematous and in a few areas showed a slight increase in fibrous tissue. Some alveoli contained pigment-filled macrophages. The liver was very congested and diffusely infiltrated by fibrous tissue which was irregularly distributed in both the portal and central areas. The spleen showed considerable hyperemia. The kidney was negative.

METHODS AND MATERIALS

For control material, the pulmonary vessels in the following cases from the Massachusetts General Hospital files were studied (all sections stained routinely with hematoxylin and eosin and elastic tissue stains): (*a*) 12 cases of latent pul-

monary embolization with subsequent cor pulmonale; (*b*) 21 cases of severe congenital heart disease in infants 2½ months of age or older; (*c*) 30 cases of severe mitral stenosis (all surgical material); (*d*) 10 cases of malignant systemic hypertension; (*e*) 33 cases without significant pulmonary or cardiac disease, varying in age from 2 days to 77 years.

Since medial thickening is seen normally in infants, newborn to about 1½ months, the Table is a rough mathematical comparison between the two reported cases, normal controls of all ages, and five congenital heart disease cases showing medial thickening. By comparing the thickness of the media with the external diameter of the vessel (excluding the adventitia), a ratio is obtained which indirectly expresses

Comparison of Luminal Sizes in Vessels of Less Than 100 μ

Case	Age	Associated Condition	Media: External Diameter Ratio (Percentage)
		Reported Cases 1 and 2	
9086	7 mo.	Primary pulmonary arterial disease	23
8653	10 yr.	Primary pulmonary arterial disease	18
		Normal Controls	
33 cases	3 mo.-77 yr.	No significant cardiac or pulmonary disease	8.7
15410	2 da.	Normal newborn medial thickening	25
15657	1½ mo.	Normal medial thickening of infant	15
		Controls With Congenital Heart Disease	
13483	2½ mo.	Eisenmenger's complex	25
9365	4 mo.	Patent ductus arteriosus with coarctation of the aorta	25
11985	5 mo.	Interventricular septal defect	27
9964	5 mo.	Cor triloculare biatriatum with transposition of great vessels	33
16073	6 mo.	Complex cardiovascular anomalies with the features of tetralogy of Fallot plus a widely patent ductus arterio us and an anomalous arterial supply to the right lower lobe by a branch of the aorta	29

the luminal size. The difficulty of measuring a sufficient number of vessels which are cut in true cross-section is realized. Special care was taken to exclude bronchial arteries from the measurements, since they are under systemic pressure and show the medial thickness of a systemic vessel. Also excluded were vessels in which the intima showed the slightest fibrous thickening which might obscure the true luminal size.

FINDINGS

The findings in this control group fall under three headings:

(*a*) *Medial Thickening.*—Medial thickening indistinguishable from that seen in the two cases here reported was found in only five cases 2½ months of age or older (Table) from the last 10,000 autopsies. All five were in cases of congenital heart disease. As a result of this medial thickening, the lumens of the vessels were significantly decreased in diameter as compared with vessels of corresponding size in normal lungs.

(*b*) *Significant Fibrous Intimal Thickening.*—Significant fibrous intimal thickening was seen in the following cases:

1. Twenty-one of 30 cases of severe mitral stenosis.
2. Twelve of 12 cases of latent pulmonary embolization.
3. Congenital heart disease.

	Case No.	Age	Abnormality
a.	13825	65 yr.	Interventricular septal defect
b.	13197	4 mo.	Transposition of great vessels
c.	16257	16 yr.	Transposition of great vessels
d.	11356	9 yr.	Large atrial septal defect with slight pulmonary stenosis
e.	11906	2½ yr.	Tetralogy of Fallot
f.	11968	14 yr.	Tetralogy of Fallot
g.	16184	16 yr.	Large defect between aorta and pulmonary artery

4. Two of nine cases of malignant systemic hypertension.
5. Nineteen of 33 cases of normal controls (all 19 over 23 years of age).

(*c*) *No Significant Medial Thickening or Fibrous Intimal Thickening.*—No significant medial or fibrous intimal thickening was seen in the following cases:

1. Four of six cases of tetralogy of Fallot (uncomplicated by other anomalies).
2. Three of four cases of patent ductus arteriosus.
3. One of two cases of pulmonic stenosis.
4. One of four cases of interventricular septal defect.
5. Seven of nine cases of malignant systemic hypertension.
6. Nine of 30 cases of severe mitral stenosis.
7. Normal controls from 2 months to 16 years of age.

COMMENT

In fetal life blood largely by-passes the lungs and enters the aorta directly through the ductus arteriosus. This results, at least in part, from an increased resistance throughout the pulmonary arterial tree, as indicated morphologically by the thick-walled small arteries and arterioles with small lumina similar to systemic vessels.[11] Toward the end of fetal life the ductus arteriosus begins to close and ever-increasing amounts of blood flow through the lungs. After birth the ductus closes entirely, and all the blood passes through the lungs. Simultaneously the lumens of the pulmonary vessels begin to dilate, so that at the end of about two months the lumina are quite large, as compared with the systemic vessels, and the walls thin. In normal pulmonary arterioles (defined arbitrarily by us and others [1] as being less than 100 μ in external diameter, excluding the adventitia) there is seldom a continuous muscle coat, and in most cases the internal and external elastic laminae are contiguous.

As noted in the Table the most striking feature in the two cases presented is the marked medial thickening in the arterioles and small arteries. This finding was not present in any of the normal controls over 2½ months of age, nor was it found in any other routine autopsy or surgical material at this hospital with the exception of the five cases of congenital heart disease in the Table. In each of these latter cases, the medial thickening was a secondary phenomenon, since the anomaly was of such a type that pressure in the systemic and pulmonary systems tended to be equalized. Medial thickening was not seen in any case in which pulmonary hypertension developed after birth, as in mitral stenosis, although this has been reported.‖ It is interesting to note that secondary medial thickening was not seen in any cases of congenital heart disease over the age of 6 months, even though some had anomalies which were identical with those in which the secondary medial thickening did appear.

‖ References 6 and 8-10.

CONCLUSIONS

It would seem, then, that in the two reported cases there was persistence of the medial thickening normally present in fetal life and that the consequent increased resistance to blood flow resulted in the pulmonary hypertension which was present clinically. The persistence of the medial thickening in the congenital heart disease cases (Table) can be explained as a compensatory mechanism in response to the abnormal amounts of blood being delivered into the pulmonary arterial tree. The increased resistance produced by the medial thickening, and the corresponding decrease in luminal size, serves to reduce the pressure in the alveolar capillaries, and where there is an anomalous shunt, as in the interventricular septal defect, to deflect some of the blood into the systemic circulation. In the two cases of primary pulmonary arterial disease the medial thickening served no apparent useful purpose, and we found nothing to explain its persistence.

One point which must be considered is whether or not the arterial medial thickening could be secondary to some defect in the bronchioles or alveoli. In Case 1 there was marked collapse of the alveoli, with overdistended alveolar ducts. However, the combination of alveolar collapse with alveolar duct distention but no medial hypertrophy was found not uncommonly in the controls. This parenchymal abnormality was noted especially in biopsy specimens from patients with mitral stenosis. Marked atelectasis was not seen in Case 2.

Another type of change which must be considered in any discussion of pulmonary arterial disease is fibrous intimal thickening. This was not seen in Case 1 (age 7 months) but was present to slight degree in Case 2 (age 10 years). This is regarded as a secondary change, resulting from pulmonary hypertension. Fibrous intimal thickening was not found to any extent before the age of 4 months and was not present to a marked degree before 16 years.

Therefore it is believed that medial thickening, from whatever cause, is present from birth and that fibrous intimal thickening is a secondary change. In almost every case showing fibrous intimal thickening there was some clearly recognizable cause for pulmonary hypertension, such as congenital heart disease, mitral stenosis, or, rarely, latent pulmonary embolization.[12] In view of the above observations, therefore, it is believed that in Case 2 the medial thickening was primary and the fibrous intimal thickening was secondary to the resulting pulmonary hypertension. It is probable that Case 1 would have developed fibrous intimal thickening had he lived beyond the age of 7 months.

SUMMARY

1. Two cases of primary pulmonary arterial disease are presented. These are characterized pathologically by marked medial thickening of the small arteries and arterioles and clinically by right-sided heart failure.

2. The findings in a number of controls are presented. These represent 30 cases of mitral stenosis, 22 cases of congenital heart disease, 9 cases of malignant systemic hypertension, and 33 normal cases of all age groups.

3. Medial thickening similar to that found in the two reported cases was present in only five of the controls. All were cases of congenital heart disease with shunts tending to produce equalization of the pressure in the systemic and pulmonary circulations.

4. In one of the two reported cases a slight amount of fibrous intimal thickening was present. The findings in the control group suggest that this is always a secondary phenomenon, and it is regarded as such in this case.

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DISTRIBUTION OF ACID MUCOPOLYSACCHARIDES AND LIPIDS IN TISSUES OF CHOLESTEROL-FED RABBITS

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PREVIOUS reports have indicated that in cholesterol-fed rabbits lipids are deposited in numerous tissues,* although attention has usually been centered on deposits in the blood vessels. The generalized nature of lipid deposits in experimental cholesterol atherosclerosis constitutes one of the major differences between this form and human atherosclerosis.† It has also been observed that the walls of normal blood vessels contain an acid mucopolysaccharide, probably chondroitin sulfate,‡ and the possibility that this substance is involved in the mechanism of cholesterol deposition in the vessels has been suggested.§ It has even been suggested that cholesterol and the acid mucopolysaccharide may enter into some sort of combination.[15] This paper describes the results of staining various tissues for mucopolysaccharides and for lipids in both normal and cholesterol-fed rabbits in an attempt to discover what relationship exists between these two groups of substances.

METHODS

One group of eight male rabbits of 2 to 3 kg. body weight was placed on a stock diet of pellets, while a comparable group of eight rabbits was placed on the same diet to which had been added cholesterol U.S.P., 1% by weight. The cholesterol was dissolved in ether and intimately mixed with the pellets, after which the ether was evaporated. After periods of three to six months on these diets the animals were killed and the tissues fixed in 10% aqueous or alcoholic formalin. Paraffin and frozen sections were prepared.

Acid mucopolysaccharide was demonstrated by the colloidal iron and Prussian blue reaction as modified by Rinehart and Abul-Haj [16] and also by metachromasia with toluidine blue (1:1,000 in phosphate-citrate buffer at pH 5.0). Sections stained with toluidine blue were allowed to differentiate in 95% alcohol for at least 30 seconds. Frozen sections were stained for lipids with Sudan black B and for cholesterol by the Schultz method. They were examined in polarized light for cholesterol crystals.

In this paper the term acid mucopolysaccharide (AMP) is used to denote all material which takes a blue color after the colloidal iron treatment and shows a rosy metachromasia with toluidine blue, except for the epithelial mucus and the granules of mast cells only. Metachromasia was found to be greatly reduced or absent after incubation in testicular hyaluronidase, after the method of Bunting.[17] On the other hand, the colloidal iron reaction was not altered by this procedure. Since the mechanism of the colloidal iron reaction is poorly understood, it cannot be

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* References 1 through 5.

† References 1 and 4.

‡ References 6 through 10.

§ References 7 and 9 through 14.

regarded as a histochemical test by Lison's criteria.[18] In spite of this, the distribution of positively stained material is the same as that of the metachromatic material, and the former method has the advantage that black and white photographs will illustrate the distribution.

OBSERVATIONS

1. *Morphological Forms of Lipid Deposits in Cholesterol-Fed Animals.*—Both by the Schultz method and by Sudan black staining three general types of lipid

*Intensity of Staining for Lipids and Extracellular Acid Mucopolysaccharide in Various Tissues**

Tissue	(1) Lipid Staining in Cholesterol-Fed Rabbits	(2) AMP Staining in Normal Rabbits	(3) AMP Staining in Cholesterol-Fed Rabbits
Heart			
Endomysium	++	+	++
Valves	++++	+++	+++
Aorta			
Intima	++++	++	++++
Media	0- +	++++	++- ++++
Muscular arteries			
Intima	++++	+	++++
Media	0- +	0- +	++
Kidney			
Glomerulus	0	++	++
Interstitial connective tissue of			
Cortex	0	+	+
Outer zone of medulla	++++	+	+- +++
Inner zone of medulla	0	++++	++++
Liver	+++	0	++
Lamina propria of			
Stomach	+++	+++	++++
Small intestine	+	++	++
Colon	0	+++	+++
Eye			
Substantia propria of cornea	0	++++	++++
Vitreous	0	++++	++++
Choroid and ciliary body	+++	+	+++
Rods and cones of retina	0	++	++
Lung			
Alveolar septa	++	0	0
Spleen			
Red pulp	+++	0	0
White pulp	0	0	0
Bone marrow (red)	++	0	0
Adrenal			
Cortex	++++	0	0
Medulla	0	0	0
Skin			
Dermis	++	++	++++
Choroid plexus	+++	0	0
Cartilage matrix	0	++++	++++
Lymph nodes	0	0	0

* 0 indicates no staining; ++++ indicates extreme staining.

deposits are recognized: (*a*) in the cytoplasm of macrophages (foam cells); (*b*) in the intercellular spaces, in either crystalline or amorphous form, and (*c*) in the parenchymatous cells of certain organs, especially the liver. It is reasonable to suppose that cholesterol, observed at any one point of time in a particular type of

deposit, may, during previous periods, have existed in the other forms. Nevertheless, when an animal is killed after several months on a cholesterol diet, most of the deposited lipid is found in the cytoplasm of foam cells. Further, there is no evidence in the sections of the existence of extracellular lipid prior to its appearance in foam cells, although probably the macrophage cells take it up from the fluids of the tissue spaces. Extracellular lipid does become apparent after foam cell masses reach a reasonably large size, at which stage some of these cells break down. This change is observed in various tissues, although it is perhaps most pronounced in the deeper parts of severe intimal lesions of the blood vessels. It seems to be related more to the large size of the foam cell mass than to any peculiarity of the tissue in which the foam cells are located. At this stage examination with polarized light reveals cholesterol crystals in the extracellular spaces.

The distribution of cholesterol in various tissues is given in the accompanying Table. An elaboration of the tabulated data appears in a later section of this paper.

2. *Acid Mucopolysaccharides in Foam Cells.*—The cytoplasm of the foam cells has the same staining reactions for AMP as the ground substance has, although frequently it stains less intensely, especially for metachromasia with toluidine blue. Metachromasia in foam cells is abolished by incubation with testicular hyaluronidase. Foam cells have essentially the same staining reaction regardless of the tissue in which they are found.

3. *Distribution of Foam Cells in Cholesterol-Fed Rabbits and Its Relation to Sites of Intense Acid Mucopolysaccharide Staining in Normal Rabbits.*—The Table (Columns 1 and 2) summarizes the relation between the distribution of foam cells in the cholesterol-fed rabbits and the degree of staining for AMP in the normal rabbits.

(*a*) Blood Vessels: The intima of the vessels of normal rabbits consists only of endothelium and a small amount of ground substance between this and the internal elastic lamina. The media of elastic-type arteries contains very intensely stained AMP, which appears to fill the spaces between the elastic lamellae. The media of muscular-type arteries contains only a very slight amount of AMP. In cholesterol-fed animals lesions of both types of arteries (and also of the large veins) consist essentially of foam cell masses lying inside the internal elastic lamina and, except in late stages, do not involve the media (Fig. 1).

(*b*) Kidney: In normal rabbits the basement membrane of the glomerulus takes a moderately intense stain for AMP. The interstitial connective tissue between the convoluted tubules and the tubules of the outer zone of the renal medulla stains faintly. The great accumulation of AMP in the kidney, however, is located in the inner zone of the medulla, between the collecting tubules, where it increases in intensity toward the apex of the pyramid.

Cholesterol-fed rabbits show no definite change in the glomerulus or in the interstitial tissue between the convoluted tubules. However, in the outer zone of the medulla great numbers of foam cells lie between the tubules, forming elongated masses radially arranged (Fig. 2). In places the foam cells break down, and cholesterol crystals and atheromatous material are seen in an extracellular location. No foam cells are observed in the interstitial connective tissue of the inner zone of the medulla, where AMP concentration is high.

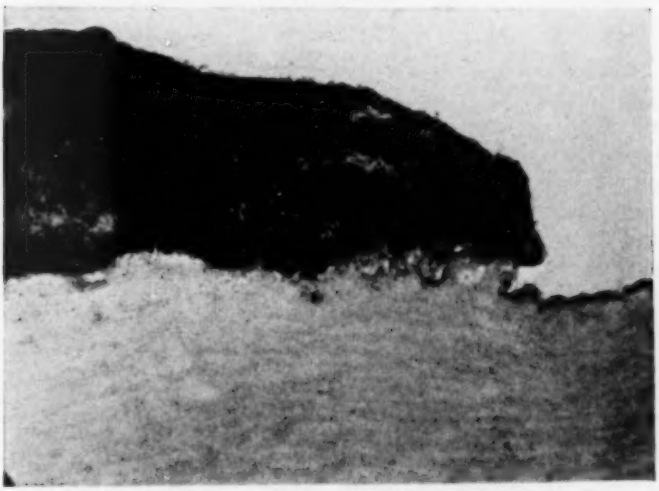

Fig. 1.—Foam cell lesion of the aortic intima in a cholesterol-fed rabbit. The lipid deposit is almost entirely confined to the intima. Sudan black B; × 100.

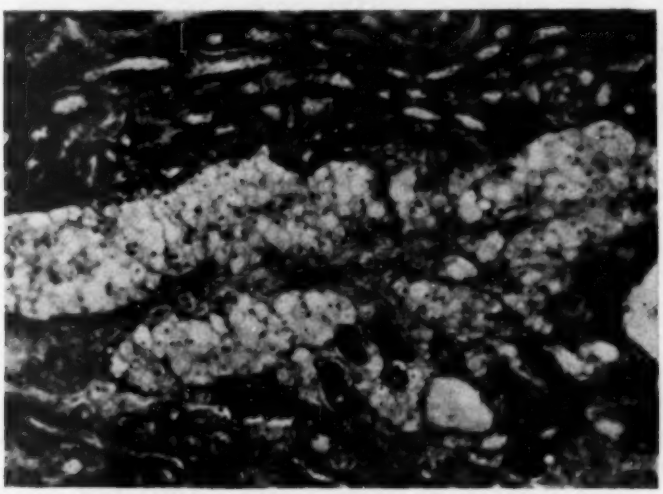

Fig. 2.—Foam cells situated between the kidney tubules of the outer zone of the renal medulla. Toluidine blue; × 120.

(*c*) Liver: Normal rabbits show almost no AMP in the liver. Cholesterol-fed rabbits develop massive accumulations of foam cells and marked lipid vacuolation of liver cells. The latter change is most marked around the central vein of the lobule.

(*d*) Lamina Propria of the Gastrointestinal Tract: The staining of the lamina propria for AMP in the normal animal is greatest in the stomach and colon. In the cholesterol-fed rabbits foam cells infiltrate the lamina propria of the stomach and small intestine only, in some cases producing greatly enlarged, club-like villi. In no animals were foam cells found in the colon.

(*e*) Eye: Although in the eye of the normal rabbit the cornea, the vitreous humor, and the layer of rods and cones of the retina are all intensely stained for AMP, while the choroid and ciliary body are only very lightly stained, it is prin-

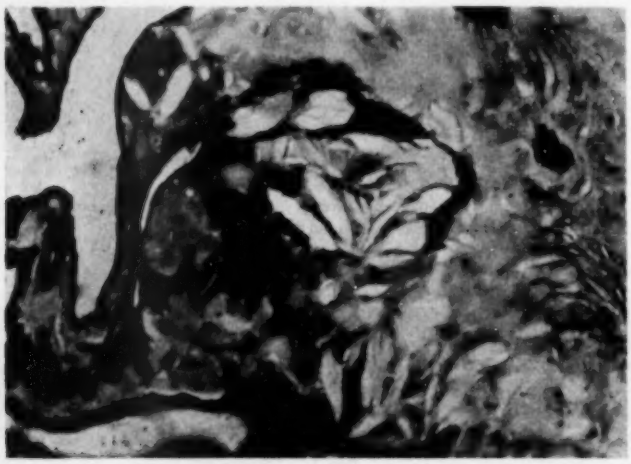

Fig. 3.—Foam cells in the ciliary body. Some of the cells are intact. Others have broken down to form an atheromatous mass, in which are cholesterol crystals. Colloidal iron reaction; × 100.

cipally the choroid and ciliary body which are infiltrated with foam cells after feeding cholesterol. The folds of the ciliary body, especially, are greatly thickened (Fig. 3).

(*f*) Skin: The normal rabbit skin displays a fairly intense staining for AMP in the delicate connective tissue surrounding the hair follicles and a much lighter staining in the papillary layer of the dermis. In cholesterol-fed rabbits foam cells infiltrate the whole of the dermis and are even scattered through the superficial subcutaneous tissue.

(*g*) Choroid Plexus of the Lateral Ventricle: The normal choroid plexus shows no appreciable staining for AMP. In cholesterol-fed rabbits foam cells appear in the delicate connective tissue under the epithelium, causing the villi to become greatly swollen.

(*h*) Spleen and Bone Marrow: These tissues in the normal rabbit contain no stainable AMP. In cholesterol-fed rabbits the red pulp of the spleen and the bone marrow are loaded with foam cells.

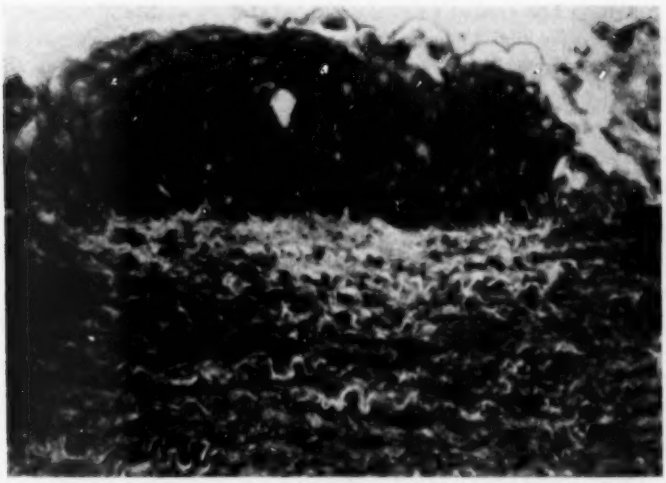

Fig. 4.—Foam cell lesion of the aortic intima, showing reduced staining for acid mucopolysaccharide in the media directly outside the intimal lesions. Colloidal iron reaction; × 100.

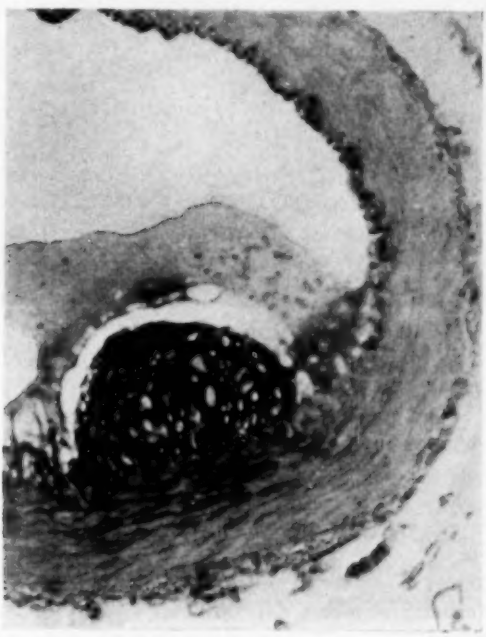

Fig. 5.—Splenic artery showing acid mucopolysaccharide in the intimal foam cell lesion and also in the adjacent media. The remainder of the media takes very little stain. Colloidal iron reaction; × 180.

(*i*) Adrenal: The normal adrenal cortex is without appreciable AMP staining. After cholesterol feeding it enlarges greatly, partly because of numerous foam cells located in the zona fasciculata and partly because of increased lipid in the parenchymatous cells.

4. *Changes in the Extracellular Acid Mucopolysaccharide (Ground Substance) in Cholesterol-Fed Rabbits.*—The Table (Column 3) gives the staining intensity of the ground substance (i. e., extracellular AMP) in the cholesterol-fed group. If this is compared with the staining intensity of the normal group (Column 2) it is seen that there is a great increase in extracellular AMP in the following tissues: the intima of blood vessels, the outer zone of the kidney medulla, and the dermis. An increase is present to a slighter extent in the lamina propria of the stomach, in the liver, in the choroid, ciliary body, and sclera, and in the endomysium and perimysium of skeletal and cardiac muscle.

Fig. 6.—Atheromatous mass in the outer zone of the renal medulla. The lesion shows foam cells, cholesterol-crystal spaces, and an intensely stained acid-mucopolysaccharide-containing ground substance. Colloidal iron reaction; × 120.

(*a*) Blood Vessels: Intimal lesions of blood vessels, from a comparatively early stage, contain an abundance of extracellular AMP. The very early lesions do not, being composed only of a few foam cells in the subendothelial region. Slightly more advanced lesions show a ringing of the foam cells with a fine layer of AMP. Late lesions show massive accumulations in their deeper parts, where fibroblastic activity is apparent.

The AMP staining of the aortic media, normally very intense, becomes greatly reduced when severe intimal lesions develop (Fig. 4). In still later lesions there is a decrease in thickness of the media. Muscular arteries, which normally have very little AMP in the media, also show atrophy of the media in late lesions. However, as the muscle fibers degenerate, abundant AMP appears in the media (Fig. 5). Thus, as far as AMP staining of the media is concerned, elastic and muscular arteries show opposite changes.

(*b*) Kidney: The only change in the ground substance of the kidney is an increase in AMP in large accumulations of foam cells in the outer zone of the medulla. As the foam cells break down and the lipid is freed into the interstitial spaces, where it may form crystals, there is a pronounced deposition of AMP (Fig. 6). Occasionally, the increased ground substance is seen in these large foam cell masses even in the absence of cholesterol-crystal formation or marked necrobiosis. No change is observed in the very abundant amount of AMP in the inner zone of the medulla.

(*c*) Skin: In the skin there is an increase in ground substance not only in the neighborhood of disintegrating foam cells but also at points where foam cells are

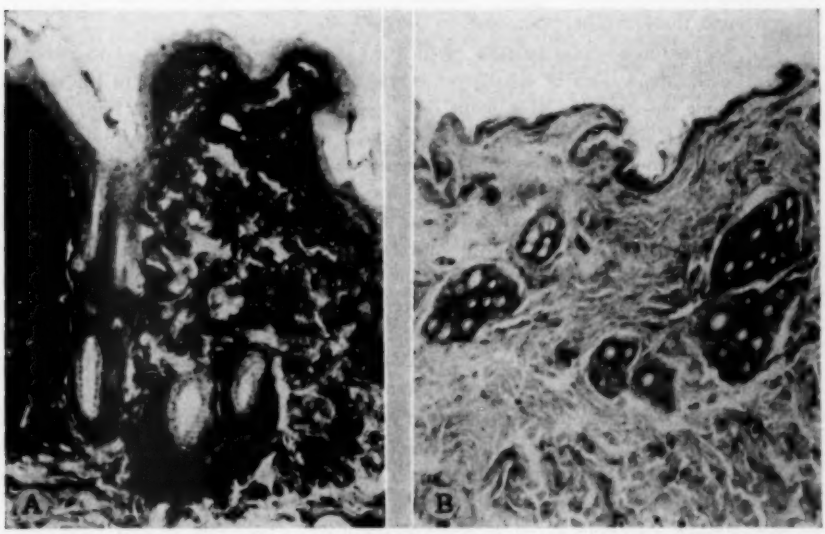

Fig. 7.—*A*, skin from the back of a cholesterol-fed rabbit. The whole dermis is intensely stained for acid mucopolysaccharide. *B*, skin from the back of a normal rabbit. Intensely stained acid mucopolysaccharide is confined to the delicate connective tissue surrounding the hair follicles. Colloidal iron reaction; × 83.

not disintegrating or are not even present. Thus, the connective tissue about the hair follicles and the papillary layer of the dermis, although usually involved in foam cell deposits (Fig. 7 *A*), shows an increased amount of AMP even when this is not the case. The skin of a normal rabbit is shown for comparison in Figure 7 *B*.

(*d*) Other Tissues: In the stomach, choroid, ciliary body, sclera, and endomysium and perimysium of muscle, extracellular AMP is associated with the breakdown of foam cells. In the liver it is associated with developing cirrhosis, chiefly about the central vein. The liver cell cytoplasm is particularly vacuolated in this region; the number of reticular fibers is increased, and AMP is a part of the ground substance about these reticular fibers.

COMMENT

With regard to the distribution of lipids in cholesterol-fed rabbits Duff [4] has made the statement that "they are precipitated in the intercellular substance of various tissues, wherever local conditions are suitable, whether in arteries or elsewhere." In the foregoing observations it was shown that few of the tissues containing large amounts of AMP undergo massive foam cell infiltration. Except for the dermis and the lamina propria of the stomach and small intestine, the sites of accumulation of great numbers of foam cells in cholesterol-fed animals are only lightly stained for ground substance in the normal animal. Therefore the amount of ground substance in a particular tissue is not in itself a factor which exerts a positive influence on the accumulation of foam cells in that tissue. To pursue Duff's hypothesis further, other "local conditions" which might influence foam cell infiltration must therefore be examined.

The degree of vascularity is a factor which must obviously be considered in relation to the deposition of substances carried in the blood. Several of the tissues which do not show appreciable foam cell deposits and which have a high normal content of AMP are avascular, or relatively so, i. e., cornea,|| vitreous humor, cartilage, and aortic media. Yet, on the other hand, the glomerular basement membrane, the inner zone of the renal medulla, and the lamina propria of the colon, which, again, do not contain foam cells after cholesterol feeding and which have a high normal content of AMP, are highly vascular.

The normal complement of fixed macrophages in a particular tissue might influence the number of foam cells seen after cholesterol feeding, since macrophages are presumably capable of extracting lipid from the sinusoidal blood and becoming foam cells. This might explain the presence of large numbers of foam cells in spleen, liver, adrenals, and alveolar septa of the lung.

Still another factor to be considered in reference to the deposition of cholesterol is the amount of tissue fluid which passes out of the capillaries and into tissue spaces. It has been shown that the lymph of cholesterol-fed rabbits is similar in lipid composition to the plasma, although the concentration of lipids is lower.[22] In most tissues lipid does not accumulate in the interstitial tissue because lymphatics carry it away. However, in certain tissues, where very great amounts of fluid are passing through capillary walls, it seems likely that if lymphatic channels exist at all, they are unable to carry away such excessive amounts of cholesterol, with the result that cholesterol accumulates in the tissue spaces, where it is taken up by macrophages to form foam cells. Such a series of events can be visualized in a tissue which produces large amounts of a plasma filtrate (e. g., ciliary body, choroid plexus); in a tissue from which large amounts of water are evaporated (e. g., lung, skin), and, possibly, in a tissue in which large quantities of serous-type secretion are produced (e. g., stomach). By comparison with the colon, the stomach is active in the production of serous-type secretion, and it will be recalled that the lamina propria of the stomach, in contrast to that of the colon, does accumulate foam cells. In the kidney, the foam cells surround mainly the loops of Henle. To my knowledge, it has not been shown whether cholesterol of hyperlipemic rabbits passes into the glomerular filtrate, although the likelihood is that at least that part of the

|| Chuma [19] found the cornea to be free of lipid infiltration, but Versé [20] and Kolen [21] have described a form of cholesterol deposit somewhat similar to arcus senilis in man.

cholesterol associated with the plasma globulin (β-lipoprotein) does not enter the filtrate. An obvious hypothesis which can explain the localization of foam cells to the region of the loops of Henle is that some of the lipid, possibly not in combination with protein, does pass into the filtrate and is resorbed in these tubules.

In glandular tissues, such as the salivary gland or pancreas, and in the renal glomerulus there is no appreciable infiltration with foam cells, although filtration or secretion is the function of such tissues. The reason may be that cholesterol is actually a constituent of the secretion or filtrate in hypercholesterolemic rabbits.

A filtration theory has frequently been advanced to explain the lipid deposits in blood vessels of cholesterol-fed rabbits.¶ It is assumed that tissue fluid containing cholesterol passes through the endothelium and that the internal elastic lamina acts as a barrier to cholesterol but not to other constituents of the filtrate. The ground substance of the media of the aorta may play an important role in the transport of water and electrolytes to the blood and lymphatic vessels of the adventitia, but there is, as yet, no experimental evidence for this function of the ground substance. It is also possible that, in addition to any function with respect to water transport, the ground substance, rather than the internal elastic lamina, may act as a barrier to cholesterol.

The changes in concentration of the AMP of the ground substance (i. e., extracellular AMP) in cholesterol-fed animals are of two types:

(*a*) The appearance of a large amount of intensely stained AMP as a result of necrobiosis of foam cells with liberation of contained AMP and lipids. The cholesterol forms crystals, and the AMP, possibly because the lipid has now separated from it, appears more concentrated than in the intracytoplasmic state. In sections it frequently outlines the crystal spaces, especially in the kidney, aortic intima, and ciliary body.

(*b*) Changes in the ground substance as a result of the activity of fibroblasts. These changes may be in the direction of an increased or of a decreased amount. In the deep part of severe lesions of the intima and in the media of muscular-type arteries there is a pronounced increase in AMP. A similar change is seen around the central vein of the liver lobule. All of these areas are characterized by collagen fibrillogenesis, and there is now abundant evidence that in active fibrillogenesis ground substance increases, as, for example, in wound healing.[24] The nature of the stimulus for collagen fibrillogenesis in areas of lipid deposition is unknown.

In view of the reduced intensity of staining for AMP seen in the media underlying severe intimal lesions of the aorta, it may be supposed that here there is a reduction in the activity of fibroblasts lying between the elastic layers of the aortic media. In the absence of any certain knowledge of the normal function of the very abundant AMP of the aortic media, it is difficult to draw any conclusions as to the means by which such a function might be disturbed by the overlying deposit of foam cells. However, as a working hypothesis, it is suggested that some "waterproofing" effect is produced by the intimal deposit so that transudation of fluid through the media is diminished, the nutrition of fibroblasts suffers, and their ability to renew AMP is impaired. In other words, atrophy of the media and decreased AMP staining may be secondary to a reduced fluid transudation through the greatly thickened intima.

¶ References 1 and 23.

It is noteworthy that similar changes have been observed in the human aorta as a result of atherosclerosis—an increased staining in fibroblastic parts of the intima and, in late stages, under severe intimal lesions, a reduced AMP staining in the media.[10]

SUMMARY AND CONCLUSIONS

As a result of cholesterol feeding, rabbits develop foam cell deposits in numerous tissues, the most striking deposition being in the intima of blood vessels, kidney, liver, dermis, spleen, lamina propria of stomach, ciliary body, choroid plexus of the brain, and lung.

No relationship can be demonstrated between the sites of high concentration of acid mucopolysaccharide in the normal animal and the sites of foam cell deposits after cholesterol feeding.

The cytoplasm of foam cells contains acid mucopolysaccharide, regardless of the tissues in which the cells are found.

The acid mucopolysaccharide of the early lesions of arteries, and also of the early lesions of other tissues, appears to be derived from foam cells.

In the later development of such lesions foam cells break down and the acid mucopolysaccharide and cholesterol are liberated, forming an atheromatous mass, in which cholesterol crystals are embedded in extracellular acid-mucopolysaccharide-containing ground substance.

Fibroblastic activity is believed to be responsible for some of the acid mucopolysaccharide seen in late lesions.

Mr. Colin G. Bower gave technical assistance.

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BILATERAL BRENNER AND KRUKENBERG TUMORS WITH OVARIAN CYSTADENOMAS

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AND
GEORGE J. RACE, M.D.
BOSTON

BRENNER tumors of the ovary are uncommon and tend to be small and unilateral.* The present predominant opinion is that Brenner tumors arise from Walthard cell rests. Greene,[4] in his recent review concerning the diverse origin of Brenner tumors, ably points out that the Walthard cell rest very likely arises from invagination of ovarian surface epithelium but that it may represent an embryonic rest in the Cohnheim sense. In addition, he presents evidence that other types of Brenner tumors may arise from the ovarian rete, the ovarian stroma primarily, or in association with pseudomucinous cystadenomata.

We have recently had a case of bilateral Brenner tumor, each ovary weighing 1,200 gm., which was associated with bilateral cystadenoma and adenocarcinoma. This case is presented because of these unique features and because it represents an interesting problem in diagnosis.

REPORT OF A CASE

A 62-year-old widowed Negro woman, mother of four children, entered the Peter Bent Brigham Hospital on June 22, 1953, with complaints of nausea and vomiting of two weeks' duration. For 18 years, firm masses, thought to be leiomyomata uteri, had been palpable in the pelvis. In the last three years, slowly enlarging, firm ovarian masses had been felt and visualized by x-ray. The menstrual history was normal, with menopause occurring at age 54, without subsequent bleeding.

On July 6 a hysterectomy and bilateral salpingo-oophorectomy were performed. The postoperative course was uneventful. One month following operation the patient was readmitted because of nausea and vomiting. Gastroscopy demonstrated a lesion, which was thought to be carcinoma, on the lesser curvature of the stomach. A subtotal gastrectomy and regional lymph node dissection were performed.

Gross Examination.—The specimen from the first operation consisted of the uterus, tubes, and ovaries. The uterus, submitted without cervix, measured 13 by 10 by 8 cm. and contained multiple 1 to 6 cm. leiomyomata, some of which were calcified. A cystic endometrial polyp, measuring 2.0 by 0.5 cm., was present. The Fallopian tubes were normal.

The ovaries each weighed approximately 1,200 gm. and were irregularly ovoid (Figs. 1 and 2). The right ovary measured 18 by 15 by 10 cm. and the left 18 by 10 by 9 cm. In each ovary the peritoneal surface was pinkish-yellow and nodular. These nodules measured 1 to 4 cm. in diameter and were firm in consistency. Except in a few peripheral cystic areas, the cut ovarian surfaces were uniformly dense and were composed of pale yellowish-white, interlacing

From the Departments of Pathology of the Peter Bent Brigham Hospital and Harvard Medical School.

*References 1 through 3.

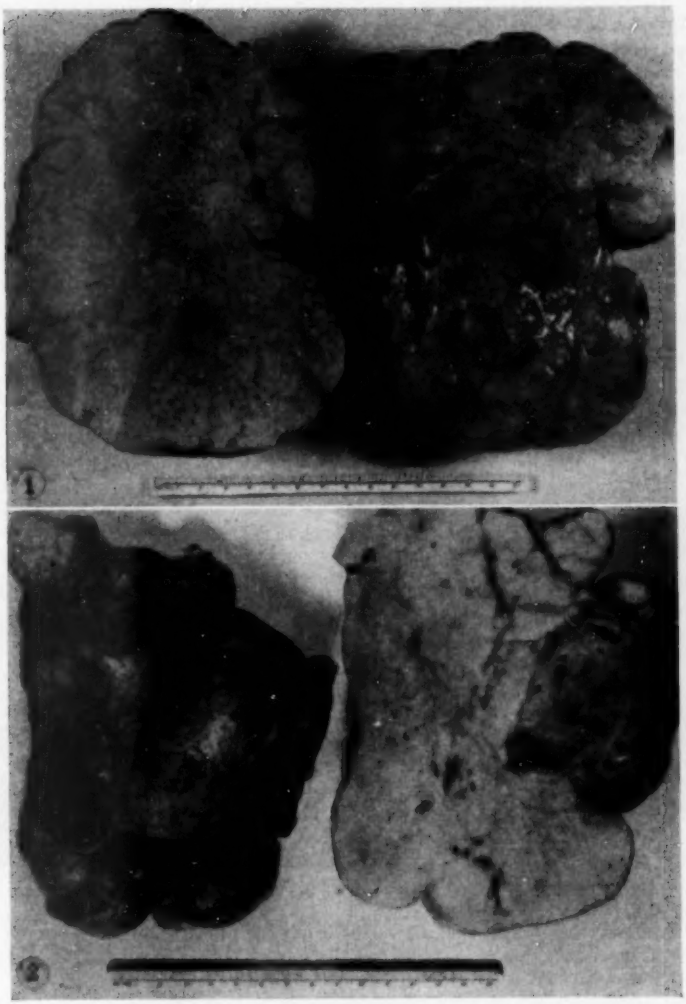

Fig. 1.—Right ovary, showing cut and external surfaces. Note the dense white cut surface, which is entirely Brenner tumor. The cystic tissue is not shown in the photograph.

Fig. 2.—Left ovary, showing cut and external surfaces. On the external surface cystadenomatous areas are represented by the dark nodules in the lower half of the photograph. On the cut surface the cystic areas represent cystadenomata, while the dense white tissue is Brenner tumor.

bundles of fibrous tissue. In the left ovary, there was an irregular peripheral cystic zone adjacent to the hilus, measuring 7 cm. in its greatest dimension, composed of 0.2 to 2.0 cm. cystic spaces, supported by soft, pinkish-gray tissue. Similar smaller areas, measuring 0.5 to 2 cm. in diameter, were visible in the hilar region of the right ovary. Focal areas of calcification were present in the solid portion of both ovaries. Approximately four-fifths of the tissue of each ovary consisted of the firm, fibrous, noncystic tumor later shown to be Brenner tumor.

The resected portion of the stomach from the second operation contained a circular ulcer, measuring 3.5 cm. in diameter, located on the lesser curvature 2 cm. proximal to the pylorus. It had the gross appearance of a typical gastric carcinoma and had penetrated the wall and metastasized to 4 of 15 regional nodes.

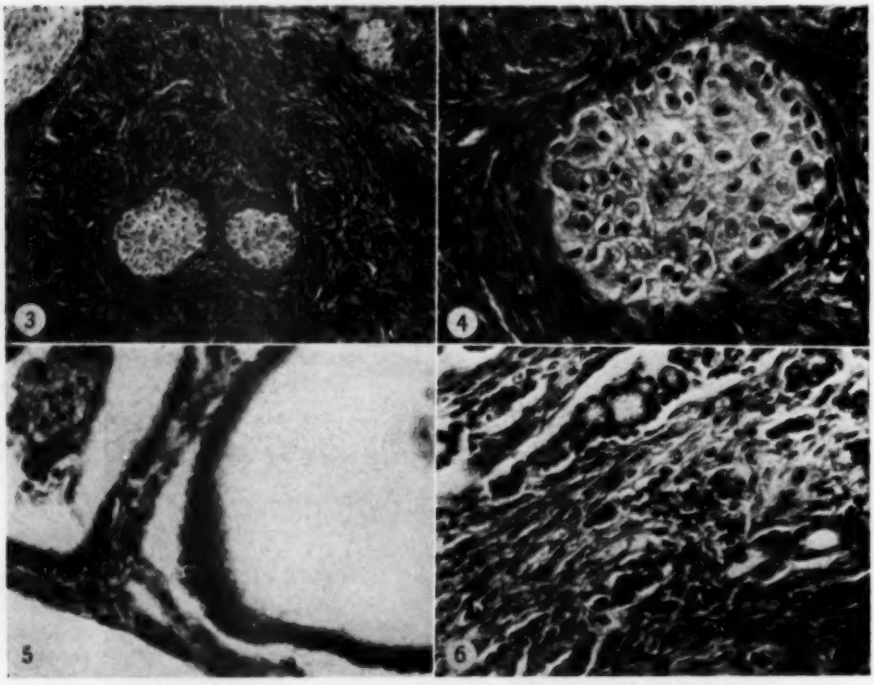

Fig. 3.—Brenner tumor, showing islands of epithelium surrounded by dense fibrous stroma. Hematoxylin and eosin; × 127.

Fig. 4.—Brenner tumor, showing details of typical Brenner epithelium. × 378.

Fig. 5.—Cystadenoma, benign, showing predominantly serous epithelium (on the left) and mucus-secreting epithelium (on the right). Hematoxylin and eosin; × 240.

Fig. 6.—Adenocarcinoma invading fibrous stroma in an area adjacent to cystadenoma (not shown). Hematoxylin and eosin; × 240.

Microscopic Examination

Bilaterally, the ovaries in the dense fibrous areas had the typical appearance of Brenner tumors (Figs. 3 and 4), with nests of epithelial cells surrounded by a dense fibrous stroma. In cystic areas (Fig. 5) well-differentiated columnar epithelium of both serous and pseudomucinous types was found lining the spaces. Within the surrounding fibrous stroma there was metastatic adenocarcinoma (Fig. 6),

similar in all respects to the adenocarcinoma of the stomach. The primary gastric carcinoma was characterized by small atypical glands, which invaded the deeper layers of the stomach wall. Mucicarmine, toluidine blue, and periodic acid-leucofuchsin stains demonstrated moderate mucin production in approximately equal quantities in the primary gastric carcinoma and in the metastatic ovarian carcinoma. Smaller amounts of mucin production occurred in the epithelium of the cystadenomas.

COMMENT

Brenner tumors of the ovary are usually benign, small, and unilateral,† and approximately one-third of them are associated with pseudomucinous cystadenomas of the ovary.[3] Before the finding of carcinoma of the stomach in this case, the ovarian carcinoma was interpreted as primary and as probably arising from the cystadenomas which were found in both ovaries adjacent to the invasive tumor. However, on comparison of the adenocarcinoma of the stomach with that in the ovaries, a marked similarity was observed, which included the secretion of mucus in approximately equal quantity by both types of cancer cells. Therefore, we interpret the ovarian adenocarcinomas as Krukenberg tumors, which are quite separate from the bilateral benign cystadenomas. The epithelium of the cystadenomas, although predominantly serous in appearance, was shown to secrete small amounts of mucin, suggesting that the cystadenomas were of a mixed type.

SUMMARY

An unusual case of bilateral multiple ovarian tumors is presented. The tumors studied were bilateral Brenner tumor, bilateral benign cystadenomas, and bilateral Krukenberg tumors. Each ovary weighed approximately 1,200 gm.; four-fifths of the ovarian tissue was estimated to the Brenner tumor. The cystadenomas were of mixed serous and pseudomucinous type. The Krukenberg tumors were secondary to an adenocarcinoma of the stomach.

Dr. Arthur T. Hertig reviewed this case.

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EFFECTS OF TEMPORARY INTERRUPTION OF RENAL CIRCULATION IN RATS

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IN A PREVIOUS study it was shown that simultaneous interruption of the circulation to both kidneys of the rat for two hours caused severe tubular necrosis leading to death in uremia in about one week.[1] In the present study a similar degree of necrosis, and probably loss of function, occurred unilaterally when only one kidney was subjected to two hours of complete ischemia. However, with the opposite intact organ maintaining life, the entire natural history of the renal lesion resulting from such ischemia could be observed.

RENAL LESIONS FOLLOWING TEMPORARY INTERRUPTION OF CIRCULATION

Adult white male rats, weighing about 200 gm. each, were used. Temporary interruption of renal blood flow was obtained by means of bulldog clamps applied to the left renal pedicle for a period of two, three, or four hours and then removed. The technique was described in detail in an earlier report.[1] Ninety-six animals were used, and these were killed for study at intervals ranging from one hour to one year.

After blood flow was restored to the kidney the organ passed through a cycle of necrosis, repair, and atrophy.

Figure 1 shows the kidney grossly at various intervals after a two-hour period of complete ischemia. The organ enlarged progressively for about one week. During the first day the medulla, especially the juxtacortical part, was much darker than the cortex (Fig. 1 *a*, *b*, and *c*). Thereafter both cortex and medulla became pale, swollen, and indistinct in structure (Fig. 1 *d* and *e*). At one week the appearance was that of an enlarged nephrotic organ (Fig. 1 *f*). Subsequently there was progressive contraction, and by three weeks the kidney was reduced to about one-third the original size (Fig. 1 *g* and *h*). By this time the usual brown color was regained. The organ then remained atrophic indefinitely.

Microscopically, necrosis was evident as early as 6 hours after removal of the clamp and became widespread and massive at 24 hours. The lesion involved the proximal convoluted tubules, especially the terminal segments. These showed coagulation necrosis of the lining cells with loss of nuclei and blockage of lumens by acidophilic debris (Fig. 2). No significant injury to the loops of Henle or to the distal convoluted and collecting tubules was discernible.

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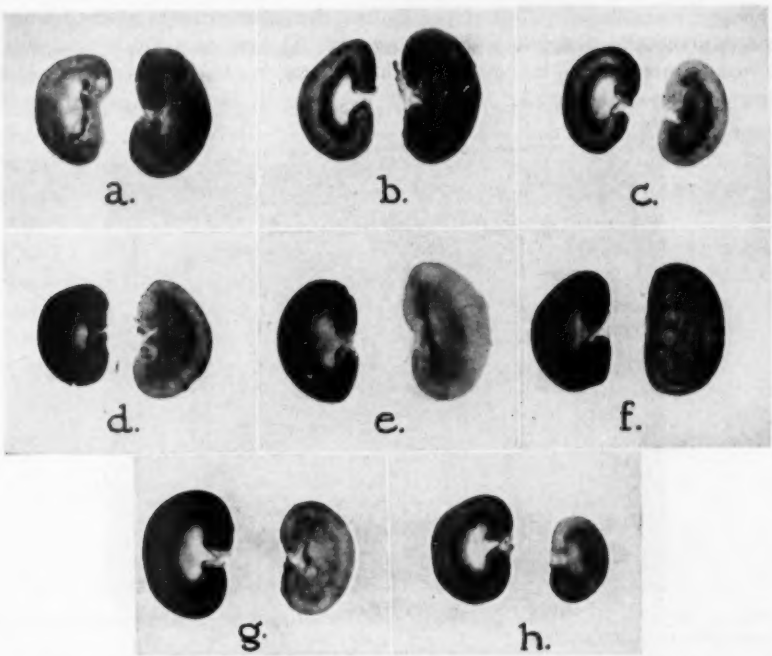

Fig. 1.—Gross appearance of injured left kidney at intervals after a two-hour period of complete ischemia. The pairs of kidneys represent the injured left organ (on the right side) and the right kidney of the same animal as control. Variation in size of the control kidneys is due largely to difference in magnification: (*a*) 1 hour; (*b*) 6 hours; (*c*) 12 hours (the left kidney is enlarged, and the right kidney is hyperemic); (*d*) 24 hours; (*e*) 5 days (note pallor of both cortex and medulla, together with indistinct structure in *e*); (*f*) 1 week (the appearance of the kidney is that of an enlarged nephrotic organ); (*g*) 2 weeks, and (*h*) 3 weeks (note progressive reduction in size of kidney).

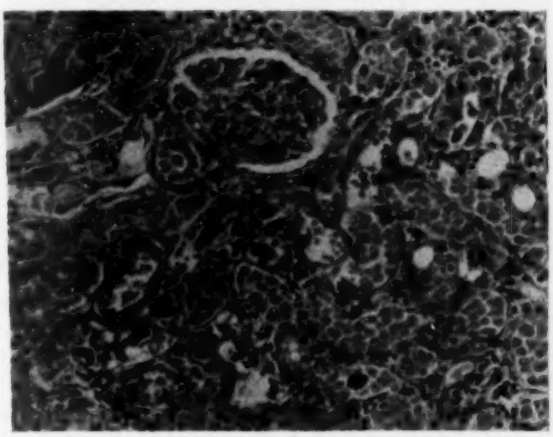

Fig. 2.—Coagulation necrosis of proximal renal tubules 48 hours after a two-hour period of complete ischemia. Hematoxylin and eosin; × 158.

Repair was usually well developed by four days after renewal of blood flow and became practically complete within two weeks. At four days the injured tubules showed new epithelial lining cells with vesicular nuclei, scant cytoplasm, and numerous mitoses (Fig. 3). Thereafter cell activity diminished, and there was

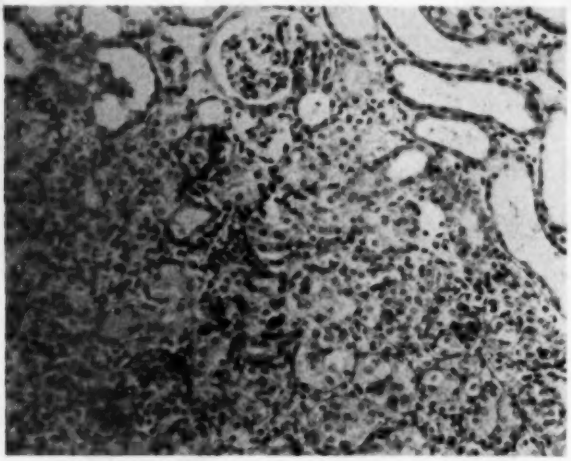

Fig. 3.—Repair of tubular necrosis four days after a two-hour period of complete ischemia. Hematoxylin and eosin; × 158.

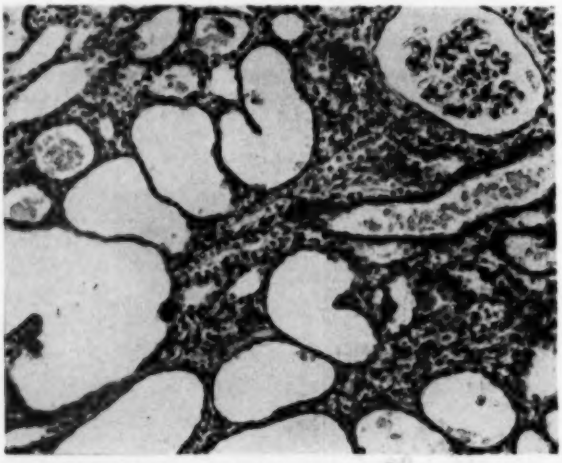

Fig. 4.—Removal of necrotic debris one week after a two-hour period of complete ischemia. Hematoxylin and eosin; × 158.

progressive clearage of necrotic material. After one week the lumens were usually empty, dilated, and lined by simple flat epithelium (Fig. 4).

Calcific deposit occurred in variable degree within necrotic tubules as early as six hours after return of circulation. At first the deposit was in a fine granular form, but later it became coarsely nodular, often filling the lumens and replacing

the necrotic debris. The deposit impeded repair, although reepithelization apparently was accomplished in some tubules by the extrusion of calcium to an interstitial position.

Casts of homogeneous colloid type were present one hour after return of blood flow and were quite numerous at six hours, filling the distal parts of the nephrons. Less frequently the casts showed interspersed pyknotic fragments or coarse basophilic material containing a few erythrocytes. After one week the number was considerably diminished. No pigment casts were observed.

During the first 24 hours after return of blood flow, some glomeruli showed edema fluid and a few erythrocytes within the capsular space. Hyperemia was not prominent. No degenerative or proliferative change was noted at any time. After one week most glomeruli were reduced in size in comparison with those in the opposite kidney.

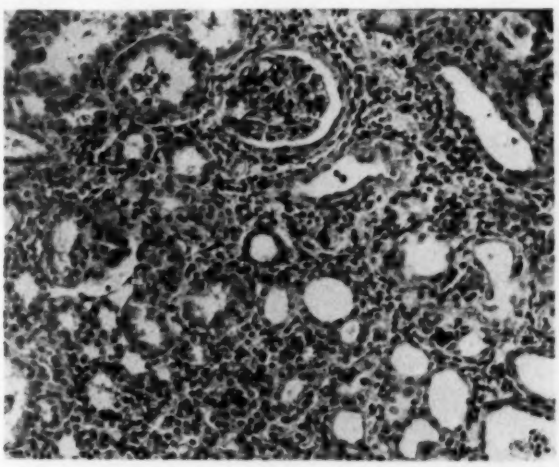

Fig. 5.—Tubular atrophy three weeks after a two-hour period of complete ischemia. Hematoxylin and eosin; × 158.

No significant lesion was detected in the renal vascular tree.

Tubular atrophy was evident about one week after renewal of blood flow and was then progressive over the second and third weeks. Most tubules were lined by small epithelial cells and had narrow or collapsed lumens (Fig. 5). After three weeks further progression of atrophy of the tubules could not be detected. Both the tubules and the glomeruli then remained atrophic permanently. There was no histologic evidence of destruction of glomeruli.

Cellular infiltrate was observed focally one day after renewal of blood flow. By one week this was moderately conspicuous, consisted mainly of lymphocytes and a small number of polymorphs, and was located interstitially in the vicinity of injured tubules. The exudate was perhaps augmented during the atrophic stage and was uniformly present thereafter in variable degree. By three weeks and thereafter polymorphonuclear cell groups were found occasionally within convoluted tubules.

No detailed morphologic study was made of the intact right kidney during the cycle of change in the opposite organ. However, the right kidney appeared to undergo slight to moderate compensatory enlargement as the opposite organ became atrophic.

COMPENSATORY HYPERTROPHY OF KIDNEY INJURED BY TEMPORARY INTERRUPTION OF CIRCULATION

The capacity of the atrophic kidney, previously injured by a period of interrupted blood flow, to undergo compensatory hypertrophy was investigated. A total of 70 rats had bulldog clamps applied to the left renal pedicle for periods of two, three, or four hours. Three weeks later, when the left kidney had become atrophic,

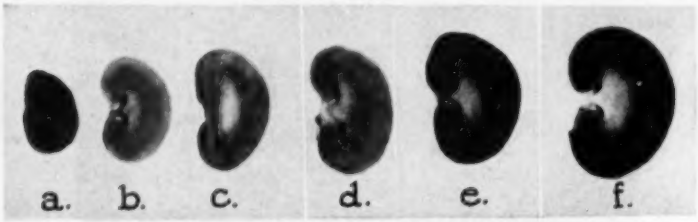

Fig. 6.—Compensatory enlargement of atrophic left kidney at intervals after resection of opposite kidney; (*a*) one day, (*b*) three days, (*c*) one week, (*d*) two weeks, (*e*) three weeks, (*f*) one month. Note progressive increase in size. Renal circulation initially interrupted for two hours.

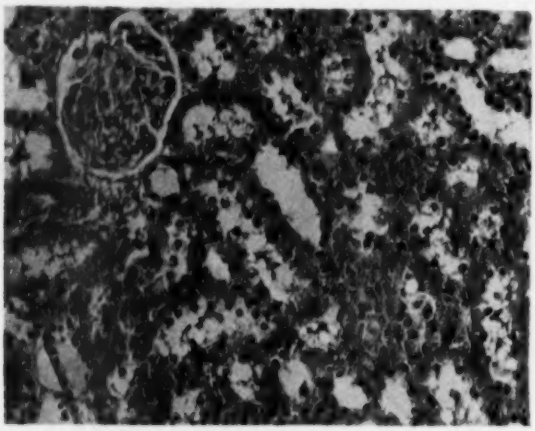

Fig. 7.—Enlargement of glomeruli and tubules one month after resection of opposite kidney. Renal circulation interrupted for two hours. Hematoxylin and eosin; × 158.

the right kidney was resected. The animals that survived were killed at periods from one day to one year later. Determinations of blood urea nitrogen and blood pressure readings by the plethysmograph technique were performed at frequent intervals.

Figure 6 shows the type of progressive enlargement that occurred in a kidney previously subjected to two hours of complete ischemia when the opposite organ was resected. At 30 days the weight exceeded that of a normal kidney and some-

times approached that of both organs. Microscopically, the great majority of nephrons participated in the enlargement (Fig. 7). The result was a uniformly enlarged kidney with fairly well-preserved cortical pattern and smooth or only slightly granular outer surface.

In the kidney of three-hour ischemia some nephrons enlarged, perhaps a mean of 50% by estimate, while the rest remained atrophic. Thus large and small nephrons occurred side by side, and the result was a moderately enlarged organ with

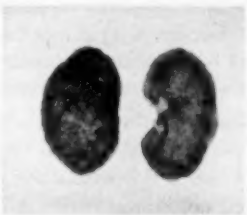

Fig. 8.—Partial compensatory enlargement of atrophic left kidney one month after resection of opposite kidney. Renal circulation interrupted for three hours.

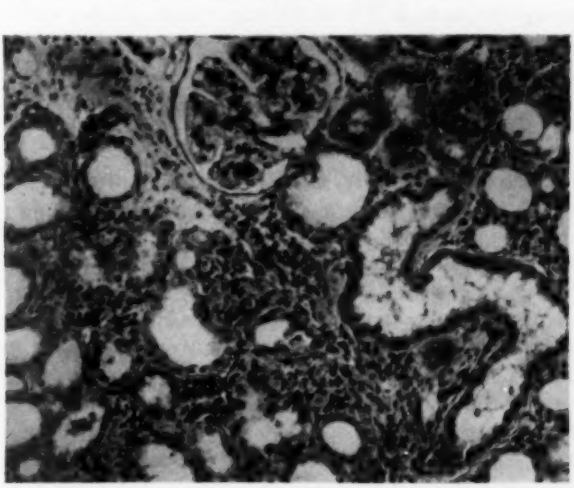

Fig. 9.—Hypertrophied and atrophic tubules in kidney with partial compensatory enlargement one month after resection of opposite kidney. Renal circulation interrupted for three hours. Hematoxylin and eosin; × 158.

distorted shape, nodular surface, and indistinct cortex (Figs. 8 and 9). Presumably the glomeruli and nephrons which failed to enlarge were those injured more severely and irreversibly by the previous period of total anoxia resulting from interruption of blood flow. In the unaltered atrophic regions there was persistence of the interstitial exudate and fibrosis.

In kidneys with a previous four-hour-period of ischemia, resection of the opposite kidney generally failed to institute compensatory hypertrophy. The great majority of nephrons, apparently injured irreversibly, in spite of repair of the initial

necrosis, remained atrophic. Hence, the organ did not increase in size, nor was its appearance significantly altered.

Functional compensation occurred more rapidly than did anatomic, since the latter usually continued for a period of about 30 days. However, in general, the extent of hypertrophy could be correlated with function and, hence, with the immediate survival of the animal. Rats with a two-hour ischemic kidney and good compensatory enlargement showed normal or only slightly elevated blood urea nitrogen and survived indefinitely. In the three-hour group, nitrogen retention varied from slight to moderate, and there was generally immediate survival and subsequent survival for a variable period, usually several months. The common course in the four-hour group was failure of hypertrophy, a rapidly rising blood urea nitrogen, and death in uremia within several days to a few weeks.

As already noted, animals with one atrophic and one intact kidney, when followed for periods up to one year after the initial renal injury usually showed no significant elevation of blood pressure. After resection of the intact kidney, the blood pressure levels generally varied according to the duration of the initial ischemia. In the majority of animals with two-hour ischemia, characterized by substantial hypertrophy and adequate renal function, the pressures remained in the normal range or were slightly elevated (140 to 160 mm.) This was also true for the majority of four-hour rats that died rapidly in uremia. However, the rats with three-hour ischemia, surviving by compensatory enlargement of one kidney without seriously compromised renal function, developed sustained hypertension (180 to 200 mm.).

COMMENT

The tubular lesion in this study was confined to the proximal convoluted segments of the nephrons. Two interrelated factors were concerned in pathogenesis; i. e., first the prolonged anoxia which induced chemical changes in the cells and then renewal of circulation which was required to complete the reaction resulting in the coagulative type of necrosis. What role may have been played by postischemic vascular spasm or possibly by vascular shunt [2] is not known. Structurally the lesion resembled the coagulation necrosis of infarction, although the latter involves entire nephrons as well as glomeruli in the area deprived of blood flow. The lesions of temporary renal ischemia and renal infarction may have a similar genesis in that each is based on interruption of blood flow and then revascularization. In infarct the latter is evidently accomplished by way of collateral channels.

Tubular necrosis as a result of temporary occlusion of the main renal artery has been described in the rat,[1] rabbit,* and dog.† Litten [3] called attention to the difference between this type of necrosis, i. e., coagulative, and the type following a permanent ligature in which rapid loss of nuclei and coagulative change do not occur but rather nuclear pyknosis and gradual dissolution of both nucleus and cytoplasm. The latter form of cell death, resulting from complete and permanent loss of blood flow to tissue and precluding collateral circulation, is basically similar to that of a resected kidney or a kidney following death of the whole organism.

In this study, the kidney showed abundant capacity for repair of the tubular necrosis. The regenerative process was like that following necrosis of tubules in

* References 3, 4, and 5.

† References 3 and 6.

mercury or uranium poisoning. Virtually complete repair was observed after periods of interrupted blood flow lasting up to four hours. Longer periods could not be studied because of the complication of renal thrombosis with resultant infarction that prevented reestablishment of circulation.

No substantial or persistent elevation of blood pressure was noted in our animals during the phase of renal necrosis or repair. Transient elevation of pressure immediately after resumption of blood flow to temporarily occluded kidneys has been reported in the dog ‡ and rat [9] and attributed to release of a pressor substance. The period immediately following removal of the renal clamp was not investigated in the present study.

Repair of tubular necrosis was followed by progressive renal atrophy. The degree of the latter was roughly proportional to the duration of ischemia even though, in some instances, it was difficult to distinguish between the small organs resulting from two- and three-hour periods or three- and four-hour periods of ischemia, respectively. Not all nephrons were involved equally in the atrophic process, even though all were reduced in size. This variation may perhaps have been due to local circulatory difference or difference in functional activity at the time the ischemia was initiated.

Atrophy would be anticipated as a probable end-result in any organ or tissue in which the blood flow was completely interrupted for a sufficiently prolonged period and then restored. However, as in complete renal ischemia, the atrophic process is likely to be preceded by swelling and necrosis of the affected part. For example, the hind extremity of a rat subjected to an occlusive five-hour tourniquet shows initial swelling and necrosis after removal of the tourniquet and, subsequently, marked atrophy of this limb if the animal survives.§

In this study, the renal atrophy was rather fully developed by the end of three weeks after removal of the constricting clamp. Very little, if any, further reduction in size was detectable beyond this time. In accord with the theory of renal counterbalance, the organ then remained permanently small and unchanged as long as the opposite intact kidney was undisturbed.[10] This had no apparent deleterious effect on the animal. Complications such as renal abscess or clear-cut pyelonephritis were not observed and, with a few exceptions, there was no hypertension or vascular disease. To what extent, if any, the atrophic kidney retained function is not known.

The experimental lesion of temporary renal ischemia has been compared with the so-called shock kidney in man. If the dog kidney is subjected to a temporary period of complete ischemia, it loses its ability to extract *p*-aminohippuric acid, largely as a result of impaired tubular function.‖ Correspondingly, the renal ischemia which occurs in man during shock as a result of low blood pressure and vasoconstriction [13] may result in such severe tubular damage that the function of the kidney is depressed when adequate circulation is restored.

There is some question as to the precise location of the renal damage in the human lesion. According to most investigators, the principal change is in the distal nephron,¶ i. e., lower nephron nephrosis, but more recently others have noted

‡ References 7, 8, and 9.
§ Unpublished data.
‖ References 11 and 12.
¶ References 6 and 14-17.

proximal tubule # and also glomerular participation.[20] With the dissection technique, Oliver [19] described a patchy disruption of both proximal and distal segments of the nephron. He believes that the proximal tubular lesion cannot be established with ordinary histologic sections, since it involves mainly the terminal segment and hence is mistaken for damage to the loops of Henle in the outer medulla. Oliver suggested the term ischemuric tubulorhexis in place of lower nephron nephrosis, since the former emphasizes both the etiologic role of reduced renal blood flow and the resulting tubular damage which is responsible for oliguria or anuria by back diffusion.

As with the human shock kidney, the morphologic lesion of experimental renal ischemia has not been altogether clarified. Using the dissection technique, Oliver [19] made a morphologic study of the kidneys from dogs subjected to complete renal ischemia for from three to six hours or to renal ischemia incident to traumatic shock. The experimental work on these animals was performed by Van Slyke and co-workers [11] and by Phillips and co-workers.[21] Oliver observed a variety of lesions ranging from coagulation necrosis of proximal tubules or coagulation necrosis combined with focal tubulorhexis to pure tubulorhexis of isolated nephrons, i. e., without associated coagulation necrosis. He therefore concluded that exactly the right degree of renal ischemia, both as to duration and extent, is required to produce the lesions of tubulorhexis in pure form. However, since the animals constituting the study were all subjected to a standard procedure, i. e., clamping of the renal artery, in order to produce "complete" ischemia, the degree of ischemia needed for the production of pure tubulorhexis in some dogs was apparently obtained by mere chance. Perhaps the variability in the degree of ischemia was due to differences in the degree of collateral circulation.

The above also applies to Badenoch and Darmody,[4] who adopted a standard procedure for occluding the renal artery in rabbits and subsequently assumed that certain animals, namely, those with distal nephron disease, had only partial occlusion. The implication that two types of lesions may result from experimental renal ischemia, namely, necrosis of proximal tubules due to temporary complete ischemia and a segmental disruptive lesion of nephrons which results from substantial reduction of blood flow rather than complete interruption, needs further study.

Our results differed in the rat. Here interruption of renal blood flow by clamping the renal artery for periods of from 30 minutes to 4 hours uniformly gave frank coagulation necrosis of proximal tubules as the essential lesion. Moreover the tubular involvement was diffuse and not randomly distributed. Since our observations were made from histologic sections, disruptive lesions and distal tubular involvement may have been overlooked, but it can be said with reasonable certainty that there was no instance of pure tubulorhexis with involvement of segments of isolated nephrons.

Oliver's report [19] and perhaps that of Badenoch and Darmody [4] constitute the only instances of the experimental production of tubulorhexis (lower nephron nephrosis) by means of direct renal ischemia. So far it has not been possible to produce fatal renal injury on the basis of tubulorhexis as the result of uncomplicated experimental shock.

References 18 and 19.

Compensatory hypertrophy has been studied extensively in the rat, especially in relation to unilateral nephrectomy,* or subtotal renal ablation.[27] In the dog Hinman [28] noted that the progressive atrophy, which usually occurs subsequent to relief of a two-week period of complete ureteral obstruction, may be prevented if the animal's opposite kidney is removed, thus placing the entire renal excretory burden on the atrophic kidney. Joelson, Beck, and Moritz [29] found that, whereas such an atrophic kidney tends to remain permanently small if its normal mate is undisturbed, it tends to enlarge and may even become hypertrophic if the other kidney is removed. Although the initiating mechanism is not clear, it is generally thought that compensatory renal hypertrophy is accomplished mainly by simple enlargement of glomeruli and nephrons.† Our studies are in accord with this view. The rapidity and extent of the hypertrophy are especially influenced by dietary factors, i. e., low protein intake retards and high protein intake accelerates enlargement.‡

In this investigation there was fairly good correlation between the degree of compensatory hypertrophy of the injured kidney and the duration of the initial ischemia. After two-hour ischemia, compensatory enlargement was diffuse, involving most nephrons, whereas after three hours it was focal, since apparently only the less injured nephrons responded to the stimulus provided by resection of the opposite kidney. In general, hypertrophy was good for the kidney deprived of blood flow for two hours, fair for the three-hour kidney, and poor or negligible for the organ with four hours of complete ischemia. Thus, the renal atrophy of two-hour ischemia proved to be reversible, of three-hour ischemia partly reversible, and of four-hour ischemia irreversible.[30]

SUMMARY

Following a temporary period of interrupted blood flow, the kidney passed through a cycle of tubular necrosis, repair, and atrophy.

The necrosis uniformly involved the proximal convoluted tubules, and no significant lesion was detected elsewhere in the nephron or in the glomeruli. In no instance did patchy tubulorhexis (lower nephron nephrosis) result from an episode of temporary complete renal ischemia.

The degree of atrophic change depended on the interval of the initial ischemia. The injured kidney remained permanently small as long as the opposite kidney was intact.

Compensatory hypertrophy and, hence, adequate renal function was readily achieved by the kidney previously subjected to a two-hour period of complete ischemia. Compensation was limited beyond this interval and ischemia of four hours' duration caused irreversible injury.

* References 22-26.

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INFANTILE PROGRESSIVE MUSCULAR ATROPHY

Value of Muscle Biopsy in the Diagnosis of and Its Differentiation from Muscular Dystrophy

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THERE is a group of idiopathic childhood disorders characterized chiefly by muscular weakness. Their clinical features are often not diagnostic, and muscle biopsies are frequently performed for the purposes of clarification. This study has been undertaken to determine the value of such biopsies.

For the purposes of discussion, these disorders may be divided into, first, those which are primary in the muscle, and, secondly, those in which the weakness is secondary to an abnormality in the central nervous system or the peripheral nerves. The primary muscle disease to be considered here is muscular dystrophy with its component subgroups: severe generalized familial muscular dystrophy, mild restricted muscular dystrophy, progressive dystrophic ophthalmoplegia, and myotonic dystrophy.[1] Those diseases of muscle secondary to central nervous system disease that are to be considered are Werdnig-Hoffmann infantile spinal progressive muscular atrophy and Oppenheim's amyotonia congenita. The peroneal muscular atrophy of Charcot-Marie-Tooth is an example of muscle disease secondary to peripheral nerve abnormality. Diseases of known etiology in which the striated muscle is affected, such as poliomyelitis, denervation, and spinal cord transection, have been used as base lines for comparison with the two above groups.

MATERIALS AND METHODS

From the autopsy files of the department of pathology of the Children's Medical Center, selection was made of all specimens in which the clinical or the pathological diagnosis was one of the following: infantile progressive muscular atrophy (which includes Werdnig-Hoffmann disease and amyotonia congenita), muscular dystrophy, and the atrophy of poliomyelitis. In addition, all muscle biopsy specimens were examined, with the exception of those with a histological diagnosis of an inflammatory process. A total of 23 autopsies and 60 surgical specimens were thus reviewed.

Material available consisted of stained slides, paraffin blocks, and wet tissue fixed in 10% formalin or Zenker's solution. The latter tissue had been stored in 80% alcohol. The original slides were used in most cases, although new sections or new blocks were occasionally required. All the muscles were stained either with hematoxylin and eosin or toluidine blue and eosin. Mallory's phosphotungstic acid hematoxylin, Mallory's aniline blue, Bodian's silver stain, Best's carmine stain for glycogen, and other stains were also occasionally used.

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RESULTS

The autopsy material was examined in order to determine the presence or absence of central nervous system changes, and thus segregate the cases of primary nervous system disease; i. e., those suffering from infantile progressive muscular atrophy. These changes, described by Conel,[2] when present, consisted of a peripheralization of the nucleus and the Nissl substance in cells irregularly distributed among the motor cells of the cord and brain stem. This early change was followed by a hyalinization and a swelling of the cell and by its terminal shrinkage and disappearance. The second phase was the most easily recognized and was relied upon most heavily (Fig. 1).

On the basis of the histological examination of the central nervous system of these 23 autopsied cases, three groups were defined. Group I was made up of 16

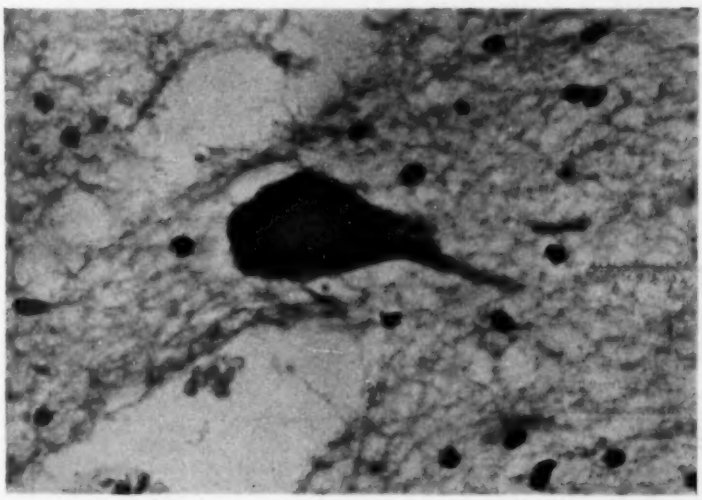

Fig. 1.—A ganglion cell from the anterior horn of the spinal cord of a case of infantile progressive muscular atrophy showing peripheralization of the nucleus and Nissl substance. Hematoxylin and eosin; × 600.

cases (69%) that satisfied the criteria of Conel.* Group II consisted of two cases (8%) in which there was a paucity of anterior horn cells, but with no active cellular degeneration. Correlation with the clinical data revealed in each of these a previous episode of poliomyelitis. No central or peripheral nervous system lesion was identified in Group III, which was composed of the remaining five cases (21%).

Examination of the muscle of the autopsied cases was performed with special attention to the presence or absence of several specific features; namely, muscle fiber size, intercellular fat, fibrosis, muscle cell degeneration, "sarcolemmal nuclear" proliferation, and myophagia. Inspection of Table 1, in which the results are tabulated, reveals certain differences in structure between these groups. Fibrosis was conspicuously absent in Group I. Interstitial fat, "sarcolemmal nuclear" prolif-

* Several of these had already been studied by Dr. Conel, and his two published cases have been included.

eration, and myophagia were less frequent in Group I than in the other groups. Muscle cell degeneration, though rare in Group I, was absent in Groups II and III. It is thus apparent that, though a tendency may exist for one of these characteristics to be present or absent, no one of these features serves as a pathognomonic sign for the identification of these groups through the histological examination of the muscle.

When the patterns of distribution of the small muscle fibers within the muscle bundle were taken into consideration, a striking difference between the groups became apparent. In the muscles of Group I there was a patchy distribution of affected

TABLE 1.—*Muscle and Ganglion Cell Changes in the Autopsied Cases*

Case No.	Clinical Diagnosis	Age, Yr.	Fiber Size: Large	Fiber Size: Small	Fat	Fibrosis	Degeneration	Sarcolemmal Nuclei Proliferation	Pattern: Myophagia	Pattern: Patchy	Pattern: Diffuse
Cases with Typical Neuronal Alterations of the Central Nervous System											
Group I *											
1	AC	3/12	−	+	+	−	−	+	−	+	−
2	AC	4/12	−	+	+	−	−	−	−	+	−
3	AC	5/12	−	+	−	−	−	−	−	+	−
4	AC	11/12	−	+	−	−	−	−	−	+	−
5	AC	3/12	+	+	+	−	+	+	+	+	−
6	AC	7/12	+	+	+	−	−	−	−	+	−
7	AC	9/12	−	+	−	−	−	−	−	+	−
8	AC	7/12	+	+	−	−	−	−	−	+	−
9	AC	5/12	+	+	−	−	−	−	−	+	−
10	AC	3/12	+	+	+	−	+	+	+	+	−
11	AC	7/12	+	+	−	−	−	+	+	+	−
12	AC	1/12	−	+	−	−	+	−	−	+	−
13	AC	4/12	+	+	−	−	−	−	−	+	−
14	AC	9/12	+	+	+	−	+	+	+	+	−
15	AC	3/12	+	+	+	−	+	+	+	+	−
16	AC	1/12	−	+	−	−	−	+	+	+	−
Cases with Paucity of Anterior Horn Cells Only											
Group II †											
17	OP	12	+	+	+	+	−	+	+	−	+
18	OP	8	−	+	+	+	−	+	+	−	+
Cases Without Abnormality of the Central or Peripheral Nervous System											
Group III ‡											
19	MD	13	+	+	+	+	−	+	+	−	+
20	MD	1 3/12	+	+	+	+	−	+	+	−	+
21	MD	12	+	+	+	+	−	+	+	−	+
22	MD	1/52	−	+	−	−	−	−	−	−	+
23	MD	9	+	+	+	−	−	+	+	−	+

* AC, amyotonia congenita.
† OP, old poliomyelitis.
‡ MD, muscular dystrophy.

fascicles within a muscle. The affected fascicles were made up of small fibers which either occupied the entire muscle bundle or, if associated with fibers of large or normal size, tended to be segregated by themselves with only a minimum of intermingling with the larger fibers (Fig. 2). In Group III, without central nervous system change, there was diffuse intermingling of fibers of all sizes within a single fascicle (Fig. 3). The cases of poliomyelitis making up Group II had diffuse intermingling of various-sized fibers within single fascicles (Fig. 4).

Since the presence of small fibers was universal in the affected muscles of the autopsy cases in all the groups, the 60 surgical specimens were reviewed, and all those biopsy specimens in which the muscle bundles contained small fibers were

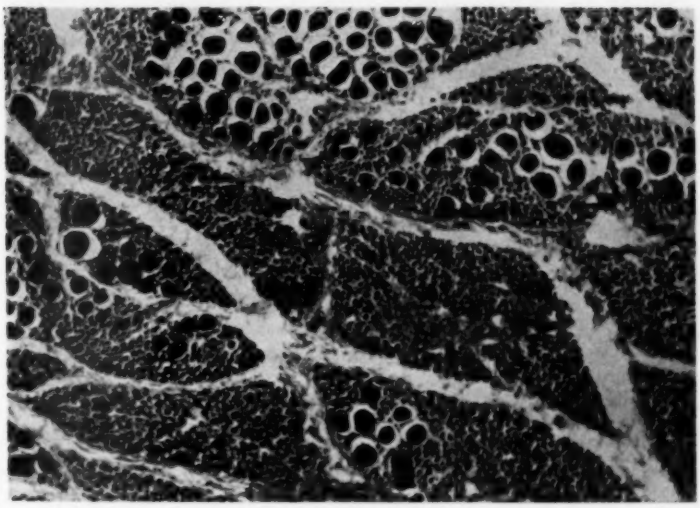

Fig. 2.—Infantile progressive muscular atrophy. The patchy distribution of fibers of different sizes is apparent in this muscle. Ganglion cell changes similar to those of Figure 1 were present. Hematoxylin and eosin; × 100.

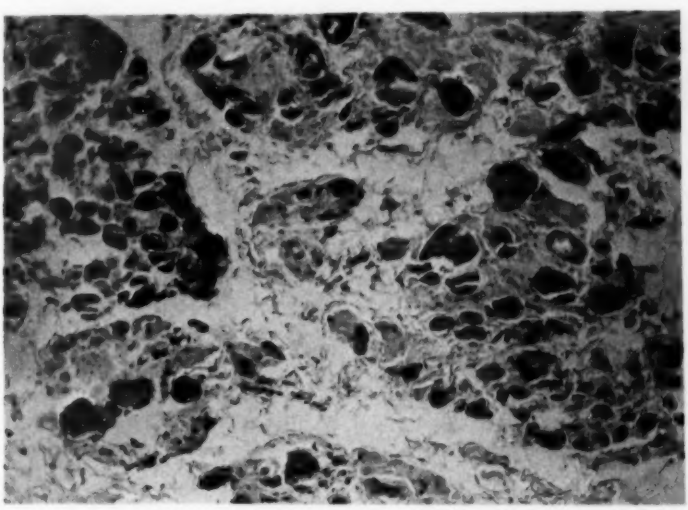

Fig. 3.—Muscle from a 13-year-old boy with muscular dystrophy. There are fibrosis, fatty infiltration, and diffuse intermingling of fibers of varying size. Hematoxylin and eosin; × 100.

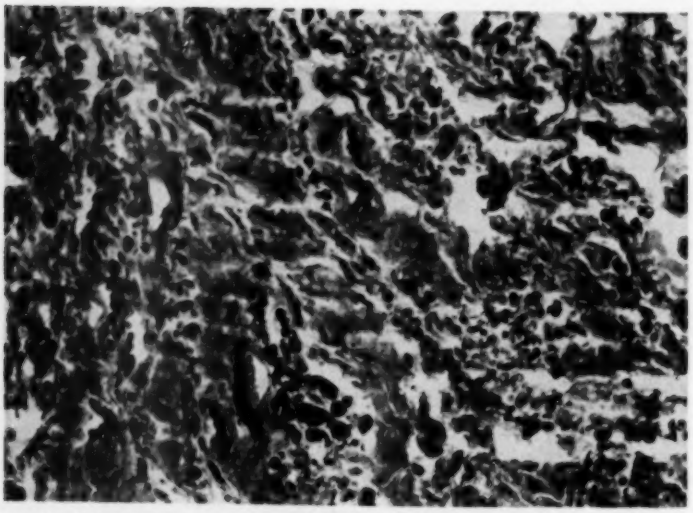

Fig. 4.—A clinical diagnosis of poliomyelitis was made four years prior to death. There is marked fibrosis with diffuse intermingling of muscle fibers of varying size. Hematoxylin and eosin; × 100.

TABLE 2.—*A Correlation of Muscle Histology with the Clinical Diagnosis in the Surgical Biopsies*

Case No.	Clinical Diagnosis	Age, Yr.	Fiber Size		Fat	Fibrosis	Degeneration	Sarcolemmal Nuclei Proliferation	Myophagia
			Large	Small					
Pattern; patchy									
1	AC	2	+	+	+	+	—	—	—
2	AC	2	+	+	+	—	—	+	—
3	AC	1 5/12	+	+	+	+	—	—	—
4	AC	1 1/12	+	+	+	+	—	—	—
5	AC	4	+	+	+	+	—	—	—
6	AC	8 5/12	+	+	+	+	—	—	—
7	AC	3 1/12	+	+	+	+	—	—	—
8	AC	6	+	+	—	—	—	—	—
9	AC	4	+	+	+	+	—	—	—
10	MD	6	+	+	+	+	—	—	—
11	CMT	2 4/12	—	+	—	—	—	—	—
Pattern; mixed									
12	AC	4 7/12	+	+	+	+	+	—	+
Pattern; diffuse									
13	MD	6	+	+	—	—	+	—	+
14	MD	10	+	—	+	+	+	—	+
15	MD	11	—	—	+	+	—	—	—
16	MD	7	—	—	+	—	+	—	+
17	MD	10	—	—	+	—	+	—	+
18	AC	7	—	—	+	+	—	—	—
19	AC	2 8/12	—	+	+	—	—	—	—
20	OP	15	+	+	+	+	—	—	—
21	OP	4	+	+	+	+	—	—	—
22	OP	11	+	+	+	—	—	—	—
23	Mm	5 9/12	+	+	+	+	—	—	—
24	PF	3 9/12	+	+	+	—	+	—	—

* AC, amyotonia congenita; MD, muscular dystrophy; CMT, Charcot-Marie-Tooth's disease; OP, old poliomyelitis; Mm, myelomeningocele; PF, pronated feet.

segregated. A total of 24 of the original 60 cases were thus selected. These 24 cases were then divided into groups, those with a patchy distribution and little or no intermingling of various-sized fibers and those in which there was a diffuse pattern with intermingling of such fibers. Thus the pattern of abnormal muscle fiber distribution was patchy in 11 specimens, diffuse in 12 specimens, and in 1 there was a patchy pattern but with intermingling of fibers of different sizes.

The biopsy specimens were also examined with regard to the presence or absence of fat, fibrosis, degeneration, sarcolemmal proliferation, and myophagia. The results of this examination are recorded in Table 2, where it can be seen that certain tendencies of the grouping of the histological features may occur, but again there is no significant correlation with the various muscle fiber size distribution patterns. A further examination of Table 2 reveals that 9 out of the 11 persons having a patchy distribution of the affected muscle bundles had a clinical diagnosis of amyotonia congenita, one of muscular dystrophy, and one of Charcot-Marie-Tooth's disease. In those cases in which a diffuse pattern of involvement was present, five had clinical diagnoses of muscular dystrophy and two of amyotonia congenita. In view of the uniformity of the patterns of muscle fiber distribution, when correlated with the presence or absence of central nervous system changes in the autopsied cases, those clinical diagnoses which are not borne out by the biopsy specimens must be regarded with suspicion.

COMMENT

The diseases described by Werdnig and by Hoffmann as infantile spinal muscular atrophy, and by Oppenheim as amyotonia congenita are identical pathologically, but are distinguished by their clinical behavior. This fact has been maintained by numerous workers since first promulgated by Greenfield and Stern.[4] Clinically, both diseases are familial and may be congenital.[5] Both have been observed in the same family group on whom a genetic study indicated to the authors that the important gene was a sublethal recessive.[6] Clinically, the patients present with hypokinesia, hypotonia, areflexia and, with an extensive symmetrical distribution of muscle weakness. The Werdnig-Hoffmann type of the disorder is more rapidly progressive than is amyotonia.[7] Classically, those persons with Werdnig-Hoffmann disease develop their difficulty after birth, have a rapidly progressive course, and die early in life. In amyotonia congenita, however, the disorder is said to be present at birth, with a slower progression and a better prognosis for the duration of life.

The use of the term infantile progressive muscular atrophy seems preferable for the disorder under discussion, even though apparent progression may not be present, and certain of the children do survive beyond infancy. In our series, in those children who were autopsied and found to have infantile progressive muscular atrophy, the range in age was 2 to 11 months with an average age of 5 months at death. Those children in whom diagnosis had been made by biopsy had ages ranging from 1 6/12 to 8 5/12 years with an average of 3 7/12 years. This distribution of ages reflects the rapid progression of one form (Werdnig-Hoffmann) in which biopsy is seldom found necessary to confirm the diagnosis.

Conel † considers the essential process in infantile progressive muscular atrophy to be one of gradually progressive degeneration of ganglion cells. The etiology of the

† References 2 and 3.

degeneration, however, is unknown. Since we have used the degenerative phenomenon described by Conel as the diagnostic feature of infantile progressive muscular atrophy, we, of course, concur with his opinion.

In histopathological descriptions of the muscles in infantile progressive muscular atrophy, there is universal agreement as to the finding of varying-sized muscle fibers. It was Brandt [8] who pointed out the peculiar fascicular distribution of large and small fibers which we have observed so consistently. Other features which have been described are an increase in fat and connective tissue and a thickening of blood vessel walls.[9] This latter feature has not been observed by us. Proliferation of "sarcolemmal nuclei" is occasionally present. Degeneration of muscle fibers accompanied by myophagia has been identified, but this is a rarely observed phenomenon.

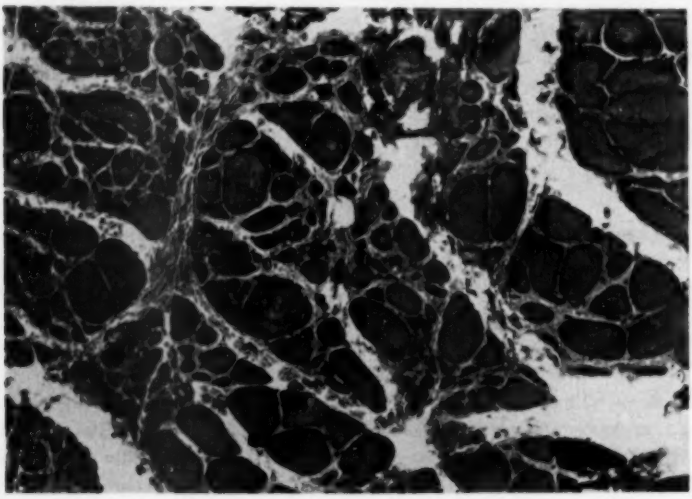

Fig. 5.—Muscular dystrophy. An electromyogram was interpreted as myotonic dystrophy. There is no tendency to segregate fibers of the same size. Compare with Figure 2. Hematoxylin and eosin; × 100.

In any given case of infantile progressive muscular atrophy, not all muscles are involved, a factor of obvious significance in interpreting a muscle biopsy. In passing, we should like to note that the diaphragm, though rarely involved, was found to be affected in one case.

According to criteria which we have accepted, the specific diagnosis of infantile progressive muscular atrophy rests on the presence of certain changes in central nervous system ganglion cells. Since the determination of the existence of these ganglion cell changes is not possible in the patient, other diagnostic methods must be used. Postmortem examination has revealed a peculiar muscle picture when ganglion cell damage was present, and, therefore, the converse will obviously be true in some, if not all, situations. As demonstrated by one case of peroneal muscular atrophy, the relationship is probably not perfect.

Of all the persons in our series who had undergone biopsy, only one (surgical Case No. 2) was followed to death, but no autopsy was obtained. In one other instance a clinical diagnosis of amyotonia congenita had been made on two brothers. The biopsy on one (surgical Case No. 15) was interpreted in our study as that of muscular dystrophy.‡ The other sibling (autopsy No. 23) had not undergone biopsy before autopsy. At postmortem examination he was clearly not a case of infantile progressive muscular atrophy from examination of the muscles as well as the central nervous system. These two cases display a clinical picture consistent with amyotonia congenita but pathologically are clearly cases of muscular dystrophy (Fig. 5).

Most of the persons with the clinical syndrome of amyotonia congenita fall into the pathological category of infantile progressive muscular atrophy. There remains a small group with the clinical picture of hypokinesia, hypotonia, and areflexia which is pathologically in the muscular dystrophy group. These can clearly be differentiated on microscopic grounds.

SUMMARY

Sixteen autopsied cases of infantile progressive muscular atrophy were studied to determine the value and accuracy of muscle biopsy in this disease. These cases were identified through the presence of a specific central nervous system ganglion cell abnormality.

The most significant feature of the histological appearance of striated voluntary muscle in the autopsied cases was the patchy distribution of small, normal, and large fibers within muscle bundles. "Sarcolemmal nuclear" proliferation, fatty infiltration, fibrosis, muscle cell degeneration, and myophagia occurred with varying frequency but were of little diagnostic help.

Criteria based on the autopsy findings were applied to a group of 24 muscle biopsies whose only common feature was the presence of small muscle fibers. With use of these criteria, and with correlation of the pathological findings with the clinical diagnosis, there were three instances in which the diagnoses did not agree.

The cases of infantile progressive muscular atrophy may be divided clinically, but not pathologically, into the Werdnig-Hoffmann type and Oppenheim's amyotonia congenita. These two types are histologically similar.

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A STUDY OF THE PATHOGENESIS OF RHEUMATIC-LIKE LESIONS IN THE GUINEA PIG

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RHEUMATIC fever is usually considered a "collagen disease" resulting from an allergic response of mesenchymal tissue to streptococci, other micro-organisms, or the products of bacterial action upon tissue. This concept is based upon such considerations as the following: (*a*) histologic changes in mesenchymal tissues with symptomatology directed primarily toward the joints and heart; (*b*) the association of rheumatic episodes following sore throat, scarlet fever, and streptococcal infections; (*c*) the similarity of symptoms and lesions to those of serum sickness, and (*d*) the tendency to recurrence of the disease and the apparent lack of immunity.

The experimental studies have been directed singularly toward the demonstration of the role of streptococci, or of allergy, or of both.* Morphologic changes have been described after anaphylactic shock [11]; after large single or repeated doses of horse,† bovine,‡ duck,[17] and pig sera § or fractions thereof, and after combinations of Streptococcus toxin and horse serum,‖ and of streptococci with scurvy.¶

The present study embraces a series of experiments intended to discover some of the many possible influences upon the production of the cardiovascular and joint changes in the guinea pig in the hope that such knowledge might (*a*) give a broader perspective to the pathogenesis of rheumatic-like lesions of the guinea pig, (*b*) disclose promising avenues for future research, and (*c*) contribute to an understanding of the pathogenesis of rheumatic fever in man.

METHODS AND MATERIALS

General Plan of Experiments.—The large number of experiments indicated in Table 1 may be divided into two categories: the specificity of the inciting agents and the "essential" influences in the mechanism of body response. For criteria of the rheumatic-like lesions, emphasis has been

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* References 1 through 10.

† References 12 through 19.

‡ References 18 through 21.

§ References 18, 19, and 22.

‖ References 23 through 25.

¶ References 26 and 27.

directed toward the morphogenesis of the lesions rather than to a single pathognomonic change, such as the Aschoff body. The evaluation of experimental results is based upon morphologic changes in the joints and cardiovascular system. At the latter site, the major lesions have been found in the cardiac valves, with alterations in the ground substance and reticular network, the endothelium, the subendothelial and diffusely scattered fibroblastic cells, and the valve rings. In the interpretation of the results, attention is drawn to the significance and relationships of the morphologic criteria.

The mechanism of body response through which the recognized changes are brought about is the most difficult to evaluate and involves the differentiation of numerous modifying influences from those which are *sine qua non*. In this sense, a study of the pathogenesis of a disease is an untangling of the interrelationships of knowledgeable influences upon those alterations regarded as criteria of the disease.

Sequence of Experiments.—With use only of subcutaneous and daily injections in young, growing guinea pigs, the experiments progressed as follows: After initial studies on injections of altered collagen and the observations of the resulting lesions,[28] the specificity of this response was tested with bacterial products and with sodium caseinate. When a nonspecificity was apparent, various substances reported by others to have produced arteritis or serous inflammation, such as allyl compounds and tyramine, were employed, along with a number of compounds, including butyl salicylate and sodium muconate. These experiments suggested a lack of chemical specificity but also disclosed that the incidence and degree of cardiac valvular changes roughly paralleled the extent of alterations at the injection site. Two influences upon the connective tissue metabolism, acute scorbutus and cortisone, were explored. Acute scurvy did not appreciably aggravate the lesions induced by bacterial products; cortisone led to a diminished metachromatic ground substance in the cardiac valves but, in larger doses, led to considerable cytologic proliferation as well. Concomitant scorbutigenic diet with cortisone also gave rise to changes, but acute scurvy prior to cortisone administration did not. The larger doses of cortisone resulted in a decrease in metachromatic ground substance while inducing considerable cellular proliferation of the valves. This was the first evidence that the several morphologic components of the cardiac valve could be altered independently of one another.

The study of histamine as a possible chemical mediator of anaphylactic hypersensitivity disclosed that lethal doses of histamine, "covered" by diphenhydramine (Benadryl) hydrochloride, U. S. P., also produced lesions. When these agents were combined with scurvy and cortisone, even severer lesions were produced. Further experiments using cortisone as an inhibitory or preventive agent have been deferred until the more general responses of connective tissues are explored.

A rough correlation between the local inflammatory response at the injection site and the cardiac valvular alterations suggests a parallelism between local and systemic responses. It is possible that local damage could alter the animal's "resistance" and produce an exacerbation of a chronic spontaneous disease. A group of animals with spontaneous streptococcal lymphadenitis were studied, and an acute infection was induced with pasteurized and with unaltered Friedländer type organisms recovered from spontaneous infection in the guinea pig. It was apparent that the chronic streptococcal inflammation in the guinea pig led to no appreciable alteration, while the acute induced Klebsiella infection produced severe changes involving both the ground substance and the cells of the cardiac valves, as well as the joints.

The rough parallelism between local response to injected materials and the cardiac valvular alterations might be due to a diffuse change in the connective tissue structure, especially its mucopolysaccharides. The host reaction to various exogenous mucopolysaccharides was therefore studied.

A number of mucoproteins and mucopolysaccharides from ganglion of man, bovine vitreous humor, the Friedländer organism, and gastric mucin were employed. Some interesting observations in relation both to joints and to cardiac valves resulted, and there was evidence that nonantigenic (as judged by anaphylaxis) mucopolysaccharides were capable of inducing such changes.

Trypsin was originally selected for use on the basis of its reputed induction of subcutaneous nodules in persons with rheumatic fever but not in others. This substance was especially interest-

ing in that it produced little swelling of the valves but a rather marked intensity of staining in the fibrillary network and ground substances of the cardiac valves, comparable to some forms of fibrinoid degeneration.

Technical Procedures.—Young guinea pigs of both sexes, weighing 200 to 300 gm., were obtained from commercial breeders and injected subcutaneously six days a week with various substances under study. Sterility, or freedom from bacterial contamination, was maintained by the usual procedures; heat-labile substances were sterilized by passage through Seitz filters. Animals were fed a commercial guinea pig diet or a rabbit chow supplemented with green, leafy vegetables. The scorbutigenic diet consisted of a rabbit chow with adequate minerals and vitamins except ascorbic acid; this diet readily induced acute scurvy in from 10 to 14 days; ample water was supplied; cages were sterilized weekly; animals were weighed each week; experiments were conducted throughout all seasons. From 15 to 20% of each group of animals were used as normal controls and killed during the course of the experiment. Table 1 indicates the size of each group receiving a specific agent. Animals were killed at intervals, usually weekly. Only a few groups were extended beyond six weeks.

Routine gross and microscopic studies were performed; the central nervous system was examined only occasionally; the knee joints and costochondral junctions were examined in all animals. Fixation was accomplished by 10% formalin, although alcohol-formalin and acetone were sometimes used, especially for the bones and hearts. After formalin fixation of the intact heart, a single transverse section was made through the organ with a sharp knife. With the proper plane of sectioning, all four cardiac chambers and the aortic, mitral, and tricuspid valves were exposed. After any remaining blood was washed away, the two halves of the heart were examined under the dissecting microscope; microsections were made of these halves. With experience, the technician may obtain microsections of the pulmonary valve by cutting the anterior block more deeply than usual.

Microsections of the heart and joints were stained with hematoxylin and eosin, Masson's trichrome stain, and toluidine blue. Other stains employed for special purposes included picric acid-fuchsin (Van Gieson), phosphotungstic acid hematoxylin, periodic acid-leucofuchsin (Hotchkiss-McManus method), colloidal iron (Hale) plus periodic acid-leucofuchsin (Ritter and Oleson [34]), or the technique of Rinehart and Abul-Haj [35] and a combined connective tissue stain (Lewis and Jones [36]).

The techniques used in the preparation of various mucopolysaccharides are indicated by the references in Table 1.

The antigenic properties of certain mucopolysaccharides were determined by the elicitation of anaphylaxis. One milligram of the substance was injected daily for 5 days, then an intravenous challenging injection of 0.1 to 15 mg. was given 14 days after the initiation of the injections. Equivocal results were obtained with the Schultz-Dale muscle contraction technique. Passive transfer studies were performed in certain animals, and several methods for precipitins were used, but all proved to be relatively unsuccessful, even when anaphylaxis could be readily produced.

EXPERIMENTAL RESULTS

The Cardiac Valves.—Of all the tissue changes, those in the cardiac valves are the most frequent and the severest; therefore, the cardiac valvular alterations are used as the primary criteria of the disease process.

Under the dissecting microscope, the heart valves of the young guinea pigs are delicate and transparent. Occasionally, there may be a minute area of thickening at one or more of the corpora arantii of the aortic valve. The induced changes in the cardiac valves are of several types: an edematous thickening, an opacity and increased firmness, a fine granularity at the line of contact, and a diffuse surface roughness. These changes are largely confined to the aortic and mitral valves. An alteration of one valve is not always accompanied by involvement of the others, and one aortic cusp may show a greater swelling than the other two cusps. In the

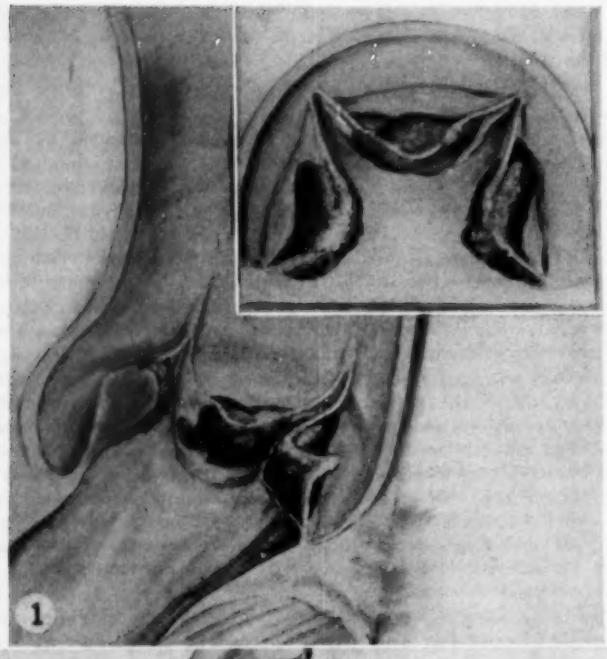

Fig. 1.—Aortic valve cusps swollen in the central, or arantal, portion. The aortic outlet is partially obstructed by the swollen cusps.

Fig. 2.—Mitral valve thickened throughout. Several coarse nodules project from the atrial aspect of the valve.

aortic valve, the edematous thickening is most prominent at or below the corpora arantii and advances out along the line of contact toward the commissures. Generally, the commissures are unchanged. In some cases, about half the aortic outlet was occupied by the thickened aortic valves (Fig. 1). In the tricuspid and mitral valves the edematous thickening is most prominent near the free border and appears as a few separate focal areas or as closely approximated areas with thin intervening grooves (Fig. 2). Thickening of the chordae tendineae may occur, but shortening has not been detected.

An accurate grading of the swelling of the valves is not possible by the methods employed in this study. The fresh heart valves, placed in nonaerated hypotonic solutions, undergo swelling in the same areas noted in formalin-fixed hearts. In the experimental animals, the more the original swelling of the valves, the greater the resulting volumetric increase. Since the degree and rate of formalin fixation will vary from animal to animal, the degree of change, if any, from the original cannot be accurately determined; therefore, the comparison of findings in Table 1 gives incidence figures based only upon the cytologic changes in the cardiac valves.

The granules along the line of contact are occasionally seen in the aortic valve but most frequently occur in the mitral valve along the medial aspect of the posterior leaflet. They frequently appear as an orderly row of minute, glistening "beads." Occasionally, a more diffuse beading is seen; this has more of the appearance of roughness, since the granules vary in size, do not occur in rows, and, generally, overlie a varying amount of thickening and opacity of the valve (Fig. 2). Beading and granularity are predominantly on those valvular surfaces that make contact and are exposed to the main current of blood, such as the auricular aspect of the mitral and the ventricular aspect of the aortic valves. A whitish opacity occurring late in the course of the experiments is due to the deposition of collagen; opacities are usually associated with focal thickening of the valve. Changes in the valve rings and bases are not generally detectable under the dissecting microscope, although a fine whitish opacity may sometimes be seen in the mitral ring.

Microscopically, the normal guinea pig valve resembles that of the human fetus. Near their attachment, the guinea pig valves contain some compactly arranged collagen fibrils and scattered, well-oriented fibrocytes. In certain areas of the mitral valve, the cardiac muscle extends in along the auricular aspects for about one-fifth the width of the valve. In the distal part of all cardiac valves of the guinea pig, there is a fine, fibrillary network of argyrophilic fibers and a few well-oriented cells of oval nuclei and sparse cytoplasm (Fig. 3). Between the reticulin and fibrocytic cells is a basophilic, homogeneous or slightly vacuolated metachromatic material, "ground substance," which is considered of an acid mucopolysaccharide composition. This stains purple with toluidine blue and blue with many of the present techniques utilizing colloidal iron.# Similar metachromatic material occurs also at the base of the aortic valve, especially at the lateral part of the mitral ring. Alcohol-formalin fixation retains more of this material than does formalin fixation alone (Fig. 4). The endothelium consists of a thin layer of cells. The dense collagen of the chordae tendineae spreads out into the delicate valve structure. With growth and aging of the guinea pig, the collagen increases in the valve; the valve always remains a thin, delicate structure without cellular proliferation.

References 34 and 35.

The description of the microscopic changes in the heart valves can be made only in reference to the recognized histologic features: quantity and staining of mucopolysaccharides; orientation, number, size, and nuclear changes in fibrocytic cells; proliferation of endothelial cells, and increase in reticulin and collagen fibers. To these may be added the location of such changes, the occasional necroses, the leucocytic infiltration, and the small hemorrhages.

The edematous thickening seen under the dissecting microscope occurs in the distal part of the mitral leaflets or in the aortic arantal area, where the "ground

TABLE 1.—*Effects of Various Agents on Cardiac Valves and Joints in Guinea Pigs*

Agent	Amount Per Day	Cardiac Valvular Lesions		Lesions in Joints
		Incidence	Severity	
Streptococcus (scarlet fever) toxin *	9,000 STD	3/10	Minimal	No change
With scorbutigenic diet	.....	2/7	Minimal	Scurvy
Diphtheria toxoid †	0.1 ml.	1/10	Minimal	No change
With scorbutigenic diet	.....	3/9	Mild	Scurvy
Tetanus toxoid ‡	0.1 ml.	2/10	Mild	No change
With scorbutigenic diet	.....	3/10	Mild	Scurvy
Staphylococcus toxoid ‖	0.1 ml.	2/9	Minimal	No change
With scorbutigenic diet	.....	4/10	Minimal	Scurvy
Sodium caseinate	60.0 mg. every 4 days	3/9	Minimal	No change
With scorbutigenic diet	.....	3/8	Minimal	Scurvy
Sodium caseinate plus diphtheria toxoid	60.1 mg. 0.1 ml.	0/5	.....	No change
With scorbutigenic diet	.....	2/5	Minimal	Scurvy
Cortisone §	1.25 mg.	2/20	Minimal	No change
Cortisone	2.5 mg.	24/39	Mild to severe	No change
Cortisone plus simultaneous scorbutigenic diet	2.5 mg.	7/15	Mild to moderately severe	Scurvy
Cortisone begun 2 wk. after scorbutigenic diet	2.5 mg.	3/18 (2/7)	Minimal	Scurvy
Methylcellulose ‖	2.0 mg.	0/10	.....	No change
Vehicle #1 for cortisone ¶	0.1 ml.	2/8	Minimal	No change
With simult. scorbutigenic diet	0.1 ml.	2/8	Minimal	No change
Histamine and diphenhydramine chloride	1.0 mg. 3.33 mg.	1/7	Minimal	No change
Histamine and diphenhydramine with cortisone-scurvy	2.5 mg.	6/8	Mild to severe	Scurvy
Tyramine	5.0 mg.	1/16	Minimal	No change
Allylamine	5.0 mg.	0/5	.....	No change
Allylformate	1.0 mg.	1/7	Very slight	No change
Allylurea	1.0 mg.	0/7	.....	No change
Sodium mucoate	25.0 mg.	2/10	Minimal	No change
Sarcosine	100.0 mg. q. 2d.	1/5	Minimal	No change
Adenosinemonophosphate ‡	0.5 ml.	2/20	Mild	No change
Butyl salicylate	0.1 ml.	3/5	Minimal	No change
Testosterone	0.1 ml.	0/14	.....	No change
Trypsin	12.5 mg.	10/18	Mild to moderate	Slight
Gastric mucin	5.0 mg.	5/16	Mild	.....
Neutral polysaccharide [39]	5.0 mg.	1/10	Minimal	Mild synov. incr. 6/10
Neutral polysaccharide [40]	5.0 mg.	3/14	Mild	No important change
Protein from neutral polysaccharide	1.0 mg.	4/15	Minimal	No change
Acid polysaccharide [39]	5.0 mg.	4/10	Minimal	Mild synov. incr. 4/10
Vitreous humor (bovine)	1.7 mg.	3/16	Minimal	Mild synov. incr. 9/16
Heat-coagulable fraction	1.0 mg.	1/8	Minimal	No change
Crude hyaluronic acid [33]	1.0 mg.	6/8	Minimal to mild	Slight synovial incr.

TABLE 1.—*Effects of Various Agents on Cardiac Valves and Joints in Guinea Pigs*—Continued

Mucin from ganglion of man	0.1 ml.	5/12	Minimal	No change
Chondroitin sulfate from cartilage of man	1.0 mg.	0/0	.....	Slight synovial incr.
Spontaneous streptococcal lymphadenitis.	.....	0/17	.....	No change
Killed streptococci of above	200,000 organisms	1/30	Very slight	No change
Friedländer's organism	1 injection 300,000 organisms	13/24	Moderate to severe	Synov. prolif. and exudate 11/24
Friedländer's organism pasteurized	300,000 organisms daily	6/12	Mild	Mild synov. prolif. 3/12
Polysaccharide from Friedländer's organism ‡‡	4.0 mg.	3/10	Minimal	Synovial prolif. 2/10

* Supplied by Lederle Laboratories Division, American Cyanamid Company.
† Commercial product, Sharp & Dohme.
‡ Commercial product, Lederle Laboratories.
§ Cortisone acetate, Merck & Company, Inc.
‖ Supplied by courtesy of Hercules Powder Company.
¶ Supplied by courtesy of Merck & Company, Inc., the vehicle for the cortisone, used as a control. It consists of 0.9% benzyl alcohol, 0.9% sodium chloride, 0.4% polyoxyethylene sorbitan monooleate, and 0.5% sodium carboxymethylcellulose in distilled water.
‡‡ Supplied through the courtesy of Ernst Bischoff Company, Inc.

substance" is most abundant. This swelling is seen first at about three days, although most of the experiments involved the killing of the first group at one week, and it is usually well seen at that time. As with the problem of accurate quantitative evaluation of the swelling of the valves, one has difficulty in judging the degree of ground substance change from a stained microsection alone; oblique sections of a valve and variations in staining intensity are some of the sources of misinterpretation. The basophilic, homogeneous or vacuolated extracellular substance stains fairly intensely with hematoxylin, toluidine blue, or colloidal iron. With cortisone, this material is diminished in quantity (Figs. 5 and 6); with trypsin, there was a distinct increase in its intensity of staining and a slight destruction of the accompanying fibrillary pattern, a picture similar to what has been termed fibrinoid (Fig. 8). The argyrophilic reticulin may increase; some collagen fibers may appear, and the small fibrocytic, or stromal, cells of the valve may enlarge; their nuclei have the serrated central chromatin bar found in the nuclei of myocytes. Their orientation is often altered, and they appear in a helter-skelter to whorled arrangement (Figs. 9 and 12).

The beaded granules seen under the dissecting microscope may be of two histologic types; both types are comprised of endothelial cells. The rarer form consists of an aggregation of endothelial cells forming a basket-like hillock of loosely arranged cells (Figs. 10 and 11). The other granules are formed of compactly arranged large cells, which extend some distance beneath, as well as beyond, the endocardial plane of the valve (Figs. 9 and 13 through 17). It is not certain whether these cells are endothelial or fibroblastic or both. No fibrils occur between the cells, and mucopolysaccharide substance is negligible. Such lesions begin at the line of contact, predominantly of the mitral valve. Later, they spread to adjoining portions of the valve, and capillaries appear but do not penetrate into the thick and central portion of the leaflet (Figs. 18 and 19). Fibrin is not encountered over the surface of such lesions. While the granules are like some verrucae, they do not have the appearance of fibrin, fibrinoid, or hyaline material seen in many verrucae of rheumatic fever in man. Palisading of the endothelial cells sometimes occurs along the surface.

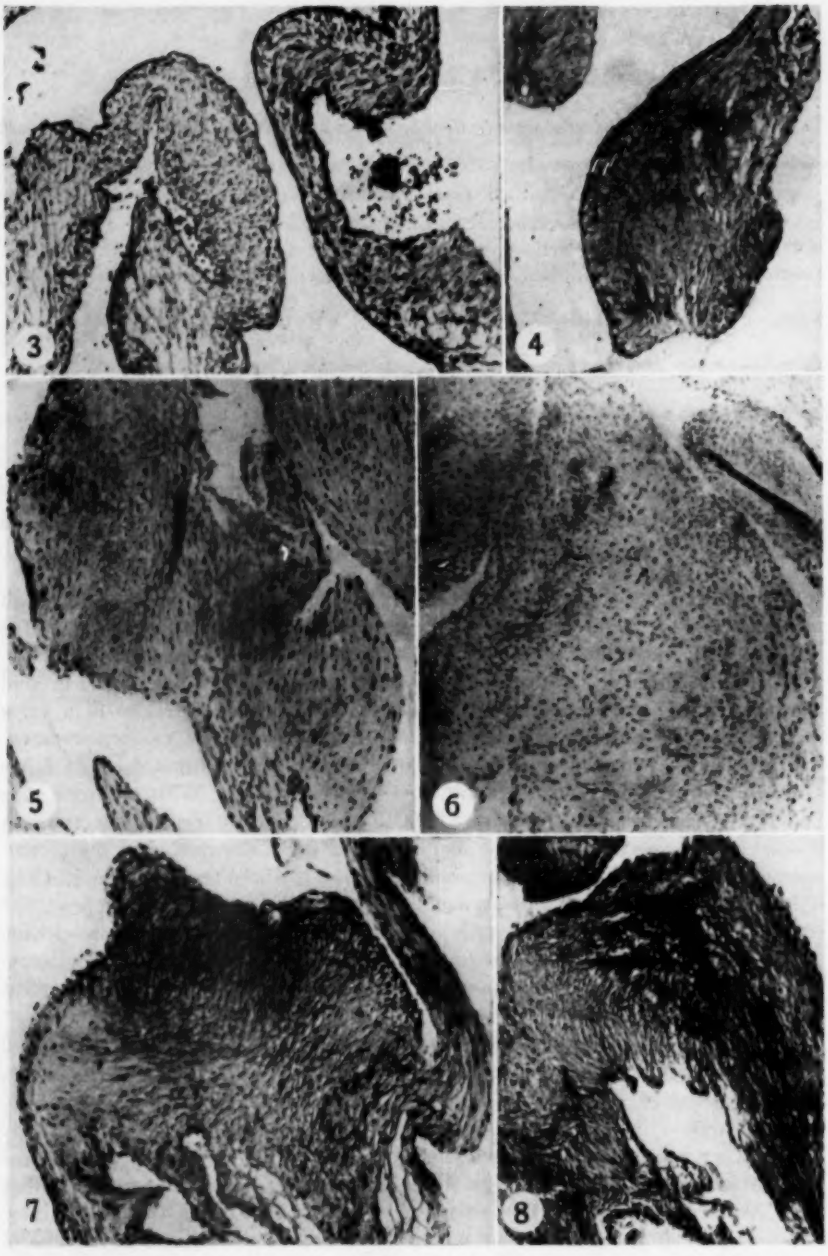

Fig. 3.—Normal mitral valve, with its delicate connective tissue fibrils, well-distributed small stromal cells, and thin endothelium. The denser collagenous portion of the valve is shown on the right. Formaldehyde fixation; hematoxylin and eosin; × 100.

Fig. 4.—Mucopolysaccharide ground substance of the mitral valve retained by alcohol-formaldehyde fixation. Toluidine blue; × 100.

Fig. 5.—Mitral valve area of cortisone-treated animal, with only a few small areas of metachromatic ground substance, shown as the darker areas in the valve. Toluidine blue; orthochromatic film; × 100.

Fig. 6.—Mitral valve area of cortisone-treated animal, with one scanty (darker) area of metachromatic ground substance. Toluidine blue; orthochromatic film; × 100.

Fig. 7.—Metachromatic substance which is denser than in Figures 5 and 6. There is also mild endothelial proliferation on the right side of the swollen portion of the mitral leaflet. Animal was given neutral polysaccharide derived from gastric mucin. Toluidine blue; orthochromatic film; × 100.

Fig. 8.—Intense metachromasia in central portion of mitral valve of animal given trypsin. Toluidine blue; orthochromatic film; × 100.

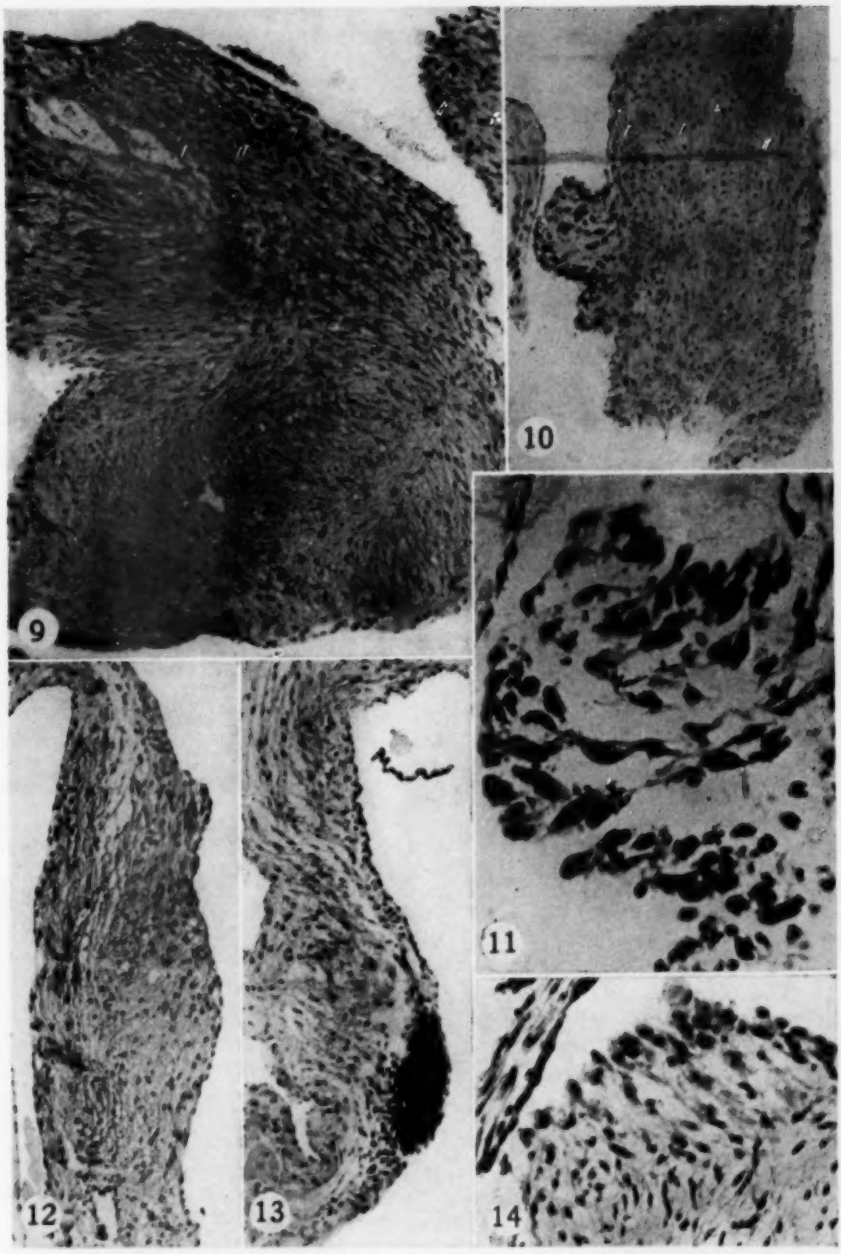

Fig. 9.—Thickened mitral valve with axial derangement of stromal cells and focal increase in endothelial and fibroblastic cells. Animal given acid polysaccharide derived from gastric mucin. Hematoxylin and eosin; × 100.

Fig. 10.—Small "hillock-like" verrucal lesion at line of contact of mitral valve, in a scorbutic animal given scarlet fever toxin. Hematoxylin and eosin; × 100.

Fig. 11.—Higher magnification of same lesions as that in Figure 10; × 350.

Fig. 12.—Small verruca at line of contact of mitral valve; slight disorganization of stromal cells. Induced infection with Friedländer type organism. Hematoxylin and eosin; × 100.

Fig. 13.—Focal increase of endothelial and fibroblastic cells, mitral valve. Induced Friedländer type infection. Hematoxylin and eosin; × 100.

Fig. 14.—Another section of same valve as that in Figure 13, showing a zone of endothelial proliferation in higher magnification. The underlying stromal fibrocytes have lost their usual orientation. Hematoxylin and eosin; × 200.

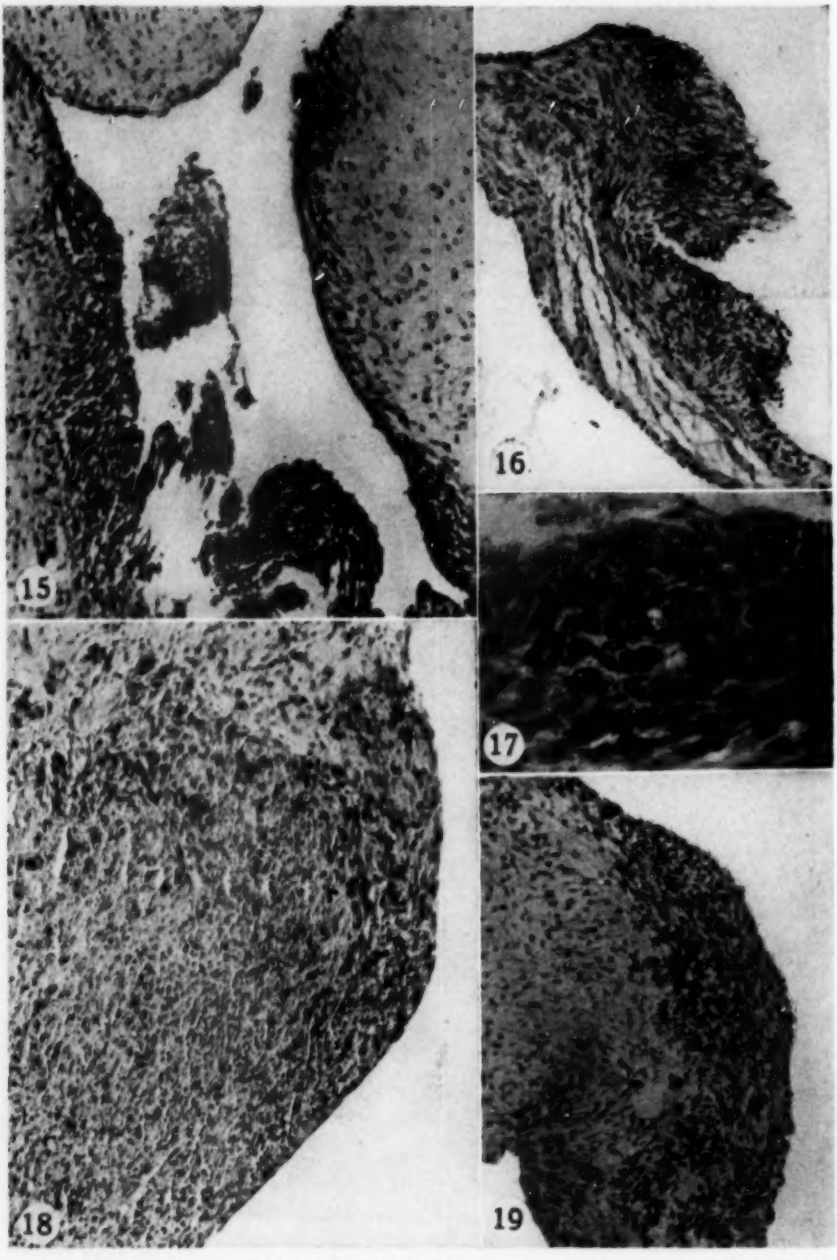

Fig. 15.—Considerable endothelial and fibroblastic proliferation on opposing, and somewhat folded surfaces of the mitral valve. Animal received diphenhydramine and histamine. Masson trichrome stain; × 100.

Fig. 16.—Brush-like proliferation of endothelial cells on atrial aspect of mitral valve. Animal given acid polysaccharide from gastric mucin; × 100.

Fig. 17. Marked proliferation of endothelial cells and fibroblasts; × 275.

Fig. 18.—One zone of proliferated fibroblasts on auricular aspect in a greatly thickened mitral valve; acute scurvy with cortisone; × 100.

Fig. 19.—Endothelial and fibroblastic cell proliferation with vascularization, mitral valve; cortisone administration only; × 100.

The base of the aortic valve may be the site of considerable edema and seems to contain a variable amount of metachromatic mucopolysaccharide. Increase in fibrillary material is uncommon, but the scattered fibrocytic cells may become more prominent. Above this part of the valve and along the wall of the aorta, a dense metachromatic substance is seen. In the lateral part of the mitral ring, myocytic cells are common and are often embedded in a metachromatic substance which is denser than that in the distal part of the valve (Fig. 23). Many fibrils may appear, and the tissue may have a hyaline-like appearance while still maintaining its metachromasia.

A few zones of necrosis which have been observed are found in the relatively unchanged mitral valve on the auricular aspect near the ring. Sometimes a few polymorphonuclear leucocytes are present; although endothelium may be absent, no fibrin precipitation is observable.

TABLE 2.—*Some Relationships of Mucopolysaccharides*

Agent	Injection Site	Cardiac Valves		Joints	Anaphylactic Sensitivity	Nitrogen, %	Hexuronic Acid, %	Hexosamine, %
		Swelling	Cell Proliferation					
Gastric mucin	Small abscesses	Moderate	Slight	No change	.....	9.3	*	9.93
Neutral mucopolysaccharide (L)	Mild macrophage storage; mild hemorrhage	Minimal	Minimal	No change	Present	6.4	3.6	16.25
Neutral mucopolysaccharide (M)	Mild macrophage storage	Minimal	Minimal	Mild synovial prolif., 60%	.....	2.8	*	14.6
Acid mucopolysaccharide	Moderate macrophage storage; mild poly. infilt.; mild hemorrhage	Minimal	Mild	Mild synovial prolif., 40%	None	1.4-4.04	16.1-24.3	6.65-17.5
Vitreous humor	No change	Moderate	None	Mild synovial prolif., 56%	.....	6.2	...	.....
Induced bacterial infection	Marked abscesses	Moderate	Moderate	Mod. synovial prolif. with mild fibrin exudation, 46%	.....	...	...	.....
Polysaccharide from above bacteria	Mild poly. and macrophage response	Minimal	Minimal	Mild synovial prolif., 20%	None	1.8	33.1	0.82

* Determination not possible because of substances interfering with the chemical test.

Histogenesis of Cardiac Valvular Lesions.—There is a general sequential relationship of the increase in ground substance, the increase in the reticulin, the proliferation of fibroblasts and endothelium, and the superficial vascularization of the involved mitral valve. There is also evidence that some of these changes may occur independently of one another.

The vascularization occurs only with considerable endothelial and fibroblastic proliferation on the auricular aspect of the mitral valve. It was seen, primarily, in animals receiving cortisone over a longer period of time than in experiments with other substances. Metachromatic ground substance may be present in the underlying valve structure but is not encountered in the focal endothelial and fibroblastic areas. Vascularization with capillaries, therefore, appears to be dependent upon the prior severer cytologic changes. Fibrosis appears late in the course of the experiments.

The increase in reticular fibrils may be unaccompanied by increase in size or number of the stromal fibroblasts. Whether these fibrils increase only after augmen-

tation of ground substance has not been conclusively demonstrated. With cortisone administration, the fibrils increase but the ground substance does not.

Perhaps the most significant relationship is between the ground substance and the cells of the valve. There is some evidence that the ground substance may be, under certain conditions, unchanged while cellular proliferation develops. The possibility of separate mechanisms for the change of ground substance and that of cells has not been explored by experiments specifically designed for this purpose but, rather, the interpretation was made from the variety of experiments performed. Cortisone administration is not accompanied by demonstrable increase in metachromatic ground substance (Figs. 5 and 6). Subcutaneous injections of trypsin intensify the fibrillary metachromatic material without producing appreciable increase in the volume of the mitral valve (Fig. 8), and gastric mucin leads to gross swelling and apparent increase in ground substance but, unlike trypsin and the higher doses of cortisone, is unaccompanied by cytologic change (Table 2). An accurate quantitative relationship between ground substance and cytologic change has been hampered by (*a*) inadequate technique for determining the volume changes in the heart valve, (*b*) the possibility of some swelling during formalin fixation and prior to examination with the dissecting microscope, (*c*) a lack of means for comparing the gross swelling with the mucopolysaccharide content other than by the metachromatic stain, and (*d*) a lack of information on factors involved in swelling of the ground substance which might, thereby, lead to a gross increase without increase in quantity of ground substance. The major histologic criterion for ground substance is its metachromasia. For this reason, a diminution in intensity of metachromasia of ground substance may erroneously be interpreted as a decrease in its quantity. Alterations in the ground substance which would cause it to imbibe fluid might not be accompanied by changes in metachromasia. Therefore, before the relation of ground substance to cytologic changes can be accurately delineated, better methods for studying the ground substance must be found.

With the technique of daily injections of foreign materials, the development of the cytologic changes appears to be progressive, and not episodic or recurrent. Experiments with spaced or interval injections have not been performed, and, as yet, no experiments have been conducted to determine whether regression might occur at any of the different phases, such as swelling of the valves or cytologic change.

Auricular Endocardium.—In a few animals, there are definite areas of proliferation of palisaded endothelial and fibroblastic cells, forming a plaque-like area in the auricular endocardium. These lesions are accompanied by valvular alterations.

Epicardium.—Proliferative changes of serosal cells are associated with pleuritis and pneumonia, as incidental to the induced infection with the Friedländer type organism (Fig. 24).

Myocardium.—The myocardial fibers show no important change. There is an increase in the basophilic material in the interstitial connective tissue. This parallels the increase in basophilic substance of the valves and is most prominent where the interstices are most pronounced, such as about the vessels. Anitschkow's myocytes occur in the interstices (Fig. 21) and at the attachments of valves. They are most frequently seen about the small myocardial arteries. The nuclei of the muscle cells of their media may resemble the myocytic nuclei, with a prominent central chro-

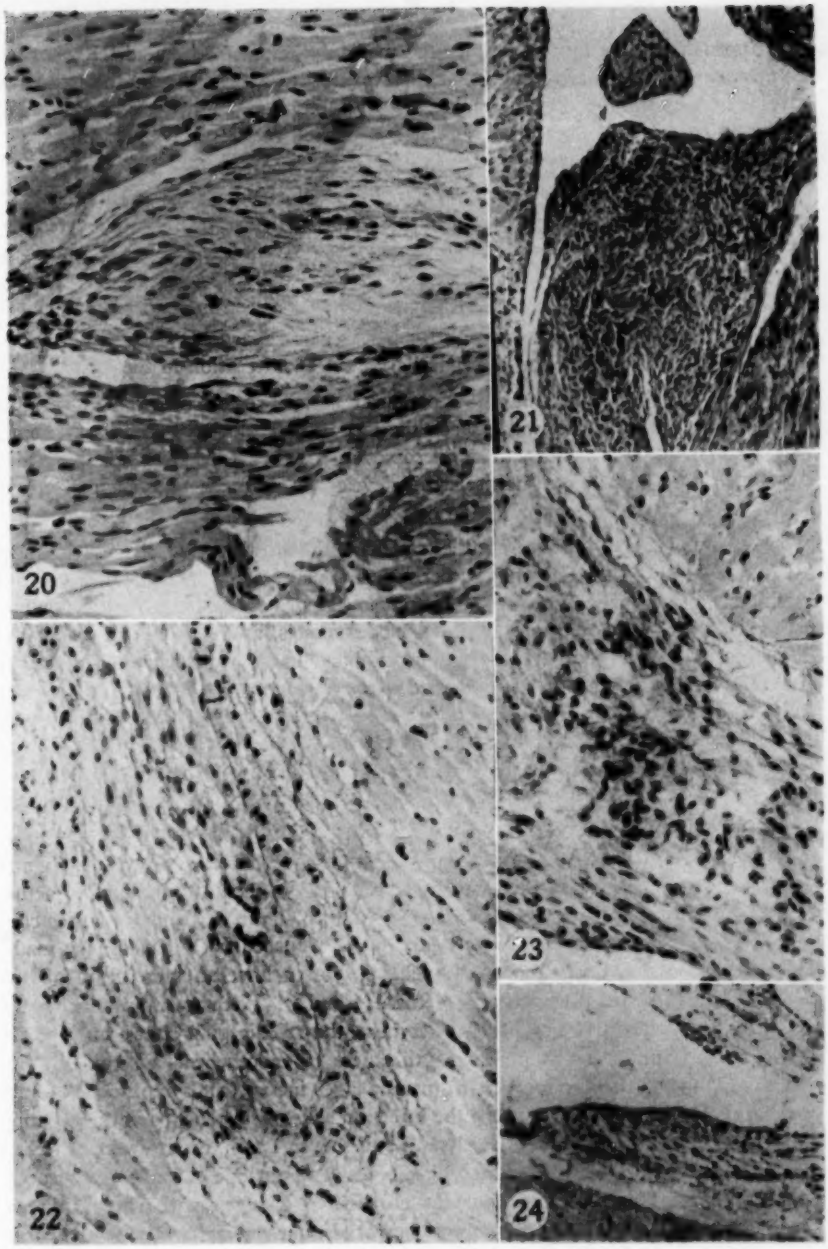

Fig. 20.—Subendocardial group of myocytes; histamine, diphenhydramine, and cortisone administration; × 200.

Fig. 21.—Marked focal proliferation of myocytes in myocardium; acute scurvy and cortisone; × 100.

Fig. 22.—Myocytes in valve ring; induced Friedländer's infection; × 200.

Fig. 23.—Coronary artery with thickened subintima; wavy zone of elastica interna; × 200.

Fig. 24.—Small area of proliferated serosal cells of epicardium; induced Friedländer's infection; × 100.

matin structure. The muscle cells of the media are often disoriented. No necroses or thromboses of vessels are seen. The nerves, most prominent near the pale myocardial cells at the top of the interventricular septum (Purkinje system), seem to increase in size; "basophilic edema" is often marked between the intermingled pale myocardial cells and the nerve fibers.

No lesions identical with the Aschoff bodies of rheumatic fever of man have been seen (Figs. 20 and 23). Groups of mononuclear leucocytes and myocytes may occur immediately beneath the endocardium of the ventricles and often near the papillary muscles. Unless these collections are unusually large or numerous, they are not regarded as significant; they may sometimes be seen in normal animals.

The endothelial cells of the capillaries are sometimes larger and more numerous, but this change does not appear to parallel any other alteration in the heart.

Aorta and Pulmonary Artery.—Most observations are confined to the ascending aorta. Metachromatic material between the medial musculoelastic lamellae becomes more prominent, along with similar increases in the cardiac valves and myocardial interstices. No fibrosis or atherosclerosis develops. Similar changes are seen in the main pulmonary artery.

Bones and Joints.—Except for the fragility of bones, the hemorrhages, the muscular atrophy, and the bulbous costochondral junctures in scurvy, no significant gross features are encountered. The histologic changes are related to the synovium, periarticular tissue, fat, tendon, muscle, blood vessels, articulating and epiphyseal cartilages, osteoid formation, calcification, and bone marrow. The changes in scurvy are well known and need not be repeated here. Changes other than those attributable to scurvy have been infrequent in our experimental studies. Some of the mucopolysaccharide substances are accompanied by definite, though mild, proliferation in synovial cells (Figs. 27 and 28; Table 2). With the Friedländer type infection, there may be a small pannus of fibrin (Figs. 26 and 29), as well as the synovial proliferation, in the joint space.

Miscellaneous Changes.—The changes resulting from the subcutaneous and intradermal injections vary from macrophage and giant cell proliferation to actual abscesses; in some instances, no appreciable reaction occurs. The spleen varies considerably in size, and no correlation with the cardiovascular lesions can be made. The animals injected with mucopolysaccharides often have an increase in plasma cells and in the hyaline fuchsinophile (Russell) bodies. With agents producing the more frequent valvular lesions such as trypsin, the splenic pulp shows an increase in pale phagocytic cells and increasing thickness of endothelized sinusoids. With age, the number of hemosiderin-laden macrophages increases. Lymph nodes sometimes appear beneath the skin about the injection sites. The changes in these lymph nodes are not remarkable. Cervical lymph nodes over 1 mm. in size were searched for and, when found, were examined histologically to exclude chronic streptococcal lymphadenitis. There are no constant correlative changes within the thymus. Adrenal glands in scurvy usually show increased relative size, and with the cortisone administration they appear to have an initial reduction in weight. The inner cortical cells tend to separate from one another in animals with scurvy and in other seriously ill animals. No distinctive or correlative changes in the liver, pancreas, bowel, lung, thyroid, skeletal muscle, or gonads were noted.

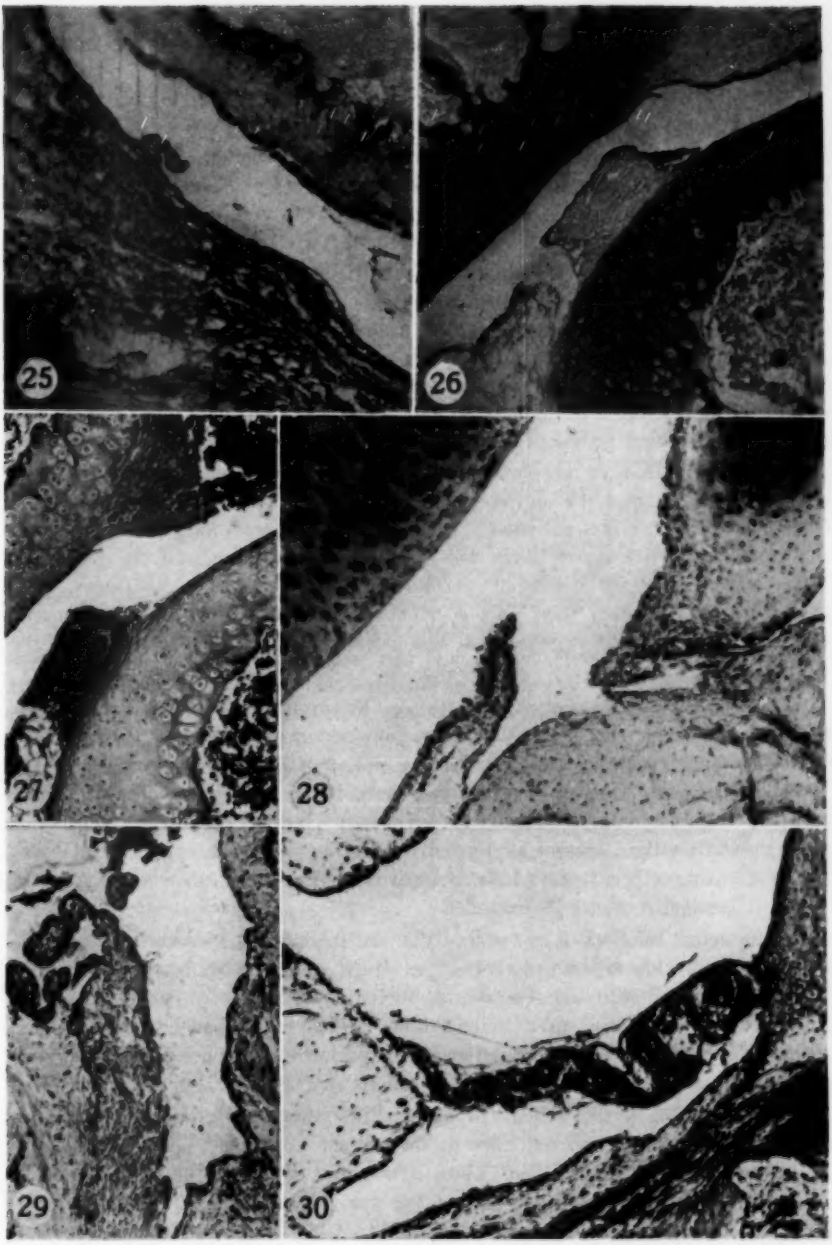

Fig. 25.—Periarticular metachromatic ground substance increased. It is shown as the dark areas in the photograph; a small mass of fibrin is present in the joint space. Induced Friedländer's infection. Toluidine blue; orthochromatic film; × 100.

Fig. 26.—Knee joint in case of induced Friedländer's infection. The pale intra-articular mass is fibrin, while the dark metachromatic synovial mucoid clings to it. Toluidine blue; orthochromatic film; × 100.

Fig. 27.—The fibrin mass in joint space of Figure 26 is more clearly shown with Masson trichrome stain; × 100.

Fig. 28.—Mild synovial proliferation: neutral polysaccharide from gastric mucin; × 100.

Fig. 29.—Mild synovial proliferation in animal given vitreous humor; × 100.

Fig. 30.—Case of arthropathy, showing fibrin in joint space. Acute scurvy and cortisone. Masson trichrome stain; × 100.

COMMENT

Morphologic Lesions.—In the heart valves of the guinea pig there is swelling of the ground substance, verrucae-like proliferations of endothelial cells, particularly at the line of contact of the mitral and aortic valves, thickening of the chordae tendineae, general thickening of the aortic and mitral valves, palisaded proliferation of the endocardial cells, and fibroblasts of the atrial endocardium. Synovial proliferation and mild fibrin deposition in the joint spaces may occur.

The Aschoff body, considered as pathognomonic of rheumatic fever in man, has not been produced in this study. Verrucae are of the cellular, and not of the hyaline or fibrin, type. Fibrinoid degeneration, although not regarded as pathognomonic, has been observed only with animals given injections of trypsin. Pericarditis or serosal cell proliferation of the pericardium, when observed, could not be attributed solely to the changes produced by the injected agents in the experimental animal.

Thus, on the basis of morphologic comparison, the only definite conclusion that seems justified is that the experimental lesion in the guinea pig and rheumatic fever in man involve mesenchymal ("collagen") tissues, with alterations in ground substance, connective tissue fibers and cells, the endocardium, and the synovium. This conclusion is not dissimilar to that of many previous investigators, each tending to regard either their own or others' experimental lesions as nonspecific (for example, fibrinoid degeneration and arteritis) or as not identical with the Aschoff body.*

Nonspecificity of the Tissue Response.—The present experiments suggest that the cardiac valvular changes in the guinea pig are not attributable to a single type of bacterium or bacterial product and are not dependent upon actual infection. These experiments further suggest that there is no specificity for such broad groups of compounds as carbohydrates or protein. Neither does there seem to be any necessity for the amino or allyl group among the simpler compounds employed, but, until many other aspects have been studied, the presence of some form of chemical specificity cannot be excluded.

While actual infection is not a requisite, the subcutaneous injections often give rise to considerable inflammatory change, which roughly parallels the degree of cardiac valvular alterations. The role of such local tissue alteration in the pathogenesis of the heart and joint lesions under consideration has not been studied adequately in the present investigation. No experiments have been conducted with other routes of administration or with injections spaced at various intervals.

The discussion of the difficult problem of the pathogenetic mechanism and its specificity is best made in reference to the possible influences upon and changes in the "target" connective tissue. Thus, scurvy and cortisone, the exacerbation of chronic or latent spontaneous infections, the mucopolysaccharides, and hypersensitivity may be considered in turn.

Scurvy and Cortisone.—It is to be expected that the general conditions or "homeostatic mechanisms" of the animal must be present before the local cardiovascular response may occur. This is suggested by the frequent absence of induced cardiovascular lesions in animals dying with an illness accompanied by marked

* References 7, 14, 27, and 37.

weight loss. Of the many possible factors which might alter the connective tissue and its responsiveness to injury, only two have been studied—cortisone and scurvy.

The smaller of the two doses of cortisone used in these experiments with guinea pigs is about half of the per kilogram clinical dose; the lesions in the guinea pig produced by this dosage were negligible. Twice this dose, or from two to four times the comparable maintenance clinical dose, led to the proliferation of fibrils and focal endothelial cells and fibroblasts in the cardiac valves. However, the duration of administration in the guinea pig may be regarded as beyond even the time limit often used in the treatment of patients, but the comparison of duration of administration to life expectancy of the species, unlike the comparison of dosage on a basis of body weight, is invalid, since the rate of cellular responses may be about the same in animals of markedly different life expectancy.

Acute scurvy has an apparent inhibitory effect upon lesions produced by the higher doses of cortisone. A simultaneous scorbutigenic diet and cortisone administration resulted in lesions, while establishment of acute scurvy two weeks prior to the administration of cortisone prevented the development of such lesions. So little is known of formation, metabolism, and enzymatic activities of connective tissue that only the more superficial speculations as to the mechanism of interaction of scurvy and cortisone are possible. Among the various speculations, the following may be mentioned: (*a*) Ground substance alterations of unspecified type may predispose to local trauma in the hearts and joints and, hence, to cytologic change; cortisone inhibits [38] and scurvy alters ground substance and its gelation qualities [39]; early developing scurvy, but not advanced scurvy, may permit the action of cortisone on formation and metabolism of ground substance. (*b*) Advanced acute scurvy produces such marked changes in the "homeostatic mechanisms" that, as in emaciation, the organism cannot respond to injury. (*c*) The adrenal gland is enlarged in scurvy and decreased in size with the initial cortisone administration; cortisone administration may alter the other adrenal functions, leading then to lesions in the connective tissue, but prior change in the adrenal from scurvy might counteract the effect of cortisone.

Some of the changes in the joints, such as those in induced infection with the Friedländer type organism, may be, in part, due to mild vitamin C deficiency. In the present experiments, ascorbic acid and alkaline phosphatase level determinations were not performed but should be done to exclude the possibility that, in such induced infections, as well as in experiments with mucopolysaccharides and cortisone, the animals were suffering from mild scurvy.

Among the many other possibilities yet to be explored in the scorbutic experiments are the additional deficiency of other unknown dietary substances, such as the antistiffness factor,[40] and disturbances in electrolytes resulting from the diarrhea of scurvy.

Latent Spontaneous Infections.—The repeated injections of "toxic" or "stressful" substances used in these experiments might produce an exacerbation of a latent or chronic infection in the guinea pig; the observed cardiovascular changes might then represent the response to such infections. On a morphologic basis, there was no evidence of exacerbation of a chronic disease; however, two spontaneous infections of the guinea pig have been investigated, the streptococcal lymphadenitis and the pneumonia and septicemia due to Friedländer's type organism.

Seventeen animals of a separate colony with streptococcal lymphadenitis showed no cytologic change in the valves, except for two which had some polymorphonuclear leucocytes. There was considerable swelling of the valves in one pregnant animal, and several others had mild swelling of the mitral and aortic valves. This, again, is an example of the mild swelling of valves in the absence of any cytologic change. Schultz [27] also failed to find any significant degree of valvular changes in animals with a similar spontaneous infection. In the present study, injection of the suspension of killed organisms isolated from the infected cervical nodes did not produce any significant cardiac valvular changes.

In our studies skin tests were not performed with any of the several streptococci implicated in this chronic lymphadenitis; however, a search was always made at necropsy for involved cervical lymph nodes. Since a chronic infection of this type does not produce cytologic change in the valve, some doubt may be cast upon its significance, unless, possibly, it is as a "preparator," in some manner, for cytologic change due to other stimuli.

The Friedländer type organism obtained from spontaneous pneumonia in a guinea pig was injected as a suspension of live organisms. Abscesses resulted at the injection site, and considerable swelling and cytologic change occurred in the cardiac valves. Many of these animals developed peritonitis, pericarditis, or pneumonia and died. With the suspension of pasteurized organisms, the local lesions were far less severe, fewer animals died spontaneously, and cardiac valvular lesions, as well as joint lesions, were milder. An acute infection from this and, perhaps, related organisms could obviate the results from any of the other experiments. There have been no studies of chronic infection with this organism or with any other bacteria or viruses.

Mucopolysaccharides.—The various mucopolysaccharides and mucoproteins used in the present study provide some information on the ability of such substances to produce changes in the joints and cardiac valves. This information, partially given in Table 1, is more clearly summarized in Table 2. Gastric mucin and vitreous humor both tend to produce swelling of the cardiac valves, but only the vitreous humor produces any synovial proliferation. One of the two neutral mucopolysaccharides, as well as the one acid mucopolysaccharide from gastric mucin, produced mild synovial proliferation, though without more than minimal swelling of the cardiac valves. Thus, there is little correlation of cardiac lesions and joint changes. The induced bacterial infection with the organism of Friedländer type led to more marked valvular and synovial changes. The acid mucopolysaccharides from this organism gave rise to mild synovial proliferation.

There appears to be no correlation of the degree of reaction at the injection site with the type of valvular change or with the proliferation of the synovia. Vitreous humor was the only substance which did not give rise to any appreciable response at the injection site. It would be of interest to study other mucoid substances which have not been chemically treated in the process of fractional isolation to see whether those rapidly entering the blood stream and producing no local response would tend to induce synovial proliferation.

The studies with antibody tracers have shown that some bacterial polysaccharides, after intravenous injection, do localize in synovia, as well as other organs,[41] but the cardiac valves have not been investigated in this manner.

Hypersensitivity.—This is the most pervasive of all hypotheses for the pathogenetic mechanism of the "collagen-vascular" diseases.

Unlike the experimental studies on serum disease, the experiments reported here were performed on guinea pigs receiving daily subcutaneous injections. These experiments were not primarily designed to test the hypothesis of hypersensitivity, but certain observations provide relevant data. Some of the findings are compatible with the theory that the injurious reaction from antigen-antibody union occurs upon cellular surfaces and not in the blood stream. The daily injections would give a continuing supply of antigen, which could unite with the "sessile" antibodies. Since the daily injections are not conducive to anaphylactic response, the antibody may still be at its site of origin or in the cardiovascular connective tissue, and not affixed to the smooth muscle. In the guinea pig, the comparatively low precipitin titers and ease of anaphylaxis may be a further indication of the "sessile" antibody and antigen combination. The lack of significant valvular lesions in the spontaneous chronic streptococcal lymphadenitis is not easily explained. The skin hypersensitivity in this disease is of the bacterial or "tuberculin" type.[42] According to one of the original delineations of this term,[43] there is, then, an absence of circulating antibody, passive transferability, and the more slowly developing skin response. But the immunity of chronically infected animals to virulent strains seems well established,[42] and the very absence of cardiovascular lesions in such animals may, therefore, be very significant. Lack of information in the nebulous concept of hypersensitivity permits only such speculations as the following: (*a*) Cardiovascular lesions require circulating antibodies or "anaphylactic" hypersensitivity; (*b*) a continuous level of antigen establishes a different state, comparable to antianaphylaxis; (*c*) the earlier responses to abnormal substances are of a different and injurious form, perhaps akin to the more rapid appearance of skin sensitivity than of circulating antibody.

Such nonantigenic substances as butyl salicylate, sodium muconate, and some of the mucopolysaccharides were capable of inducing lesions. The mucopolysaccharides capable of eliciting anaphylaxis produced the fewer joint and cardiovascular lesions (Table 2). This would argue against anaphylactic hypersensitivity as the pathogenetic mechanism were it not for the possibility that anaphylaxis is a poor criterion for anaphylactic hypersensitivity in the guinea pig. The state of "anaphylactic hypersensitivity" has been confused with the actual occurrence of anaphylactic response. Apparently, anaphylactic hypersensitivity may exist without either the occurrence or the elicitability of anaphylaxis. There is often a lack of correlation between anaphylaxis and the lesions with foreign serum.[44]

It is further possible that the substances which are apparently nonantigenic, so far as the production of anaphylaxis is concerned, may act as proantigens which form antigens at the site of the local injection by combination with some connective tissue product or by being "processed" within macrophages. In this way, one might also explain the rough parallelism between the degree of local tissue change and the cardiovascular alterations. Even the action of cortisone might be accounted for upon this basis. One might speculate further that some of the autoantibodies against the connective tissue products might combine with related substances in tissue elsewhere, especially those particularly rich in similar substances or those which might be subjected to a greater degree to "physiologic trauma."

Histamine released by the antigen-antibody union has been considered a possible chemical mediator of anaphylaxis; the manifestations of anaphylaxis in the guinea pig are caused primarily by smooth muscle contraction, suggesting that the "sessile" antibodies may be attached to the smooth muscle. If the lesions are due to hypersensitiveness, some form of response other than anaphylaxis must be operative. One of the many possibilities is that histamine could act upon other tissues, such as the connective tissue and capillaries, which might not be influenced by the "antihistamines." In the present study, lethal doses of histamine "covered" by diphenhydramine produced only mild lesions. Since many other nonlethal conditions produced far severer lesions, it is probable (*a*) that histamine is not important in the genesis of the lesions, or (*b*) that antihistamine compounds prevent or impede the development of lesions, as has been stated to occur in the development of serum disease, vascular lesions, and the Arthus reaction in rabbits by Smull and associates,[45] but denied by Dammin and Bukantz.[44] These local injections of material into the skin may be the source of considerable histamine, for products having certain features of histamine have been isolated from the skin after the injections of various agents by Kellaway.[46] If, then, the cardiovascular and joint lesions are caused by the released histamine, diphenhydramine should be effective in preventing the lesions from all the injected agents. Thus far in our experiments, we have not used diphenhydramine except in association with agents which would produce a demonstrable anaphylactic response.

Trypsin, given intravenously to nonsensitized animals, elicits anaphylactoid response proportional to the weight of the injected substance and not to the enzymatic activity.† Such anaphylactoid responses are inhibited or diminished by diphenhydramine. When trypsin is injected subcutaneously, anaphylactoid responses do not occur, and, therefore, diphenhydramine was not used in the experiments. The lesions, both at the site of injection and in the cardiovascular system, are severer than those with the histamine plus diphenhydramine experiments. Again, one cannot refute the possibility of some form of hypersensitivity in the pathogenesis of cardiovascular lesions, and, again, one is attracted to the rough parallelism of local tissue response to the cardiovascular lesions.

In some animals, the changes in the valvular ground substance occurred as early as three days after the initiation of the experiments. In induced infection with the Friedländer type organism, the valvular lesions developed before any anticipated antibody, either circulating or tissue; this suggests a more direct, or "inflammatory-toxic," response than would be associated with hypersensitivity. However, these cardiac valvular lesions may be a counterpart of the early preanaphylactic cutaneous reactions which Dienes [47] considers the first manifestation of active immunization.

Generalization.—The lesions of the connective tissue structures of the heart and joints represent a distinct, but nonspecific, morphologic entity. They are distinct in that they occur at sites (*a*) remote from the introduction of the injurious agent or (*b*) in contact with no greater concentration of the agent than that in other tissues. This remarkable responsiveness is nonspecific in that it is induced in different animal species with a variety of foreign substances and experimental techniques.

† Jones, R. S.; Carter, Y., and Rankin, J. de W.: Unpublished observations.

The mechanism through which the recognized histologic changes are brought about is unknown. To attribute these lesions to such poorly understood processes as stress or hypersensitivity is illusory. It is deemed more desirable to regard the lesions as a manifestation of the connective tissue response to injury. The responses of the connective tissue have a prominent role in the body defenses. The mechanisms of local responses at the site of injury remain as obscure as the systemic responses of fever, leucocytosis, malaise, and "aches and pains"—and as obscure as the rheumatic-like lesions under consideration.

Detailed classifications on a descriptive basis are useful, but the understanding of these body responses, antibody formation, and hypersensitiveness, and their interrelationship awaits elucidation.

SUMMARY

A series of experiments designed to discover some of the many influences upon the cardiac valvular and joint lesions in the guinea pig are presented.

Included in these experiments are a wide variety of substances injected subcutaneously; administration of combinations of some of the substances, such as cortisone and histamine; an induced infection; spontaneous streptococcal lymphadenitis, and scorbutus.

The major criteria for the experimental disease in the guinea pigs are morphologic—the cardiac valvular and joint lesions. Under the dissecting microscope, the focal swelling of the cardiac valves and the coarse and fine granules, or "verrucae," at the line of contact of the mitral valve are readily seen. Although there appears to be a general sequential relationship of the microscopic changes in the valves (swelling of the ground substance, focal proliferation of endothelium and fibroblastic cells, diffuse proliferation of stromal fibroblasts, fibrosis, and vascularization), the changes in the ground substance mucopolysaccharides can be influenced independently of the cellular proliferation.

Cortisone, in higher doses than employed clinically in man, inhibits the appearance or alters the metachromasia of the valvular mucopolysaccharides but induces cellular proliferation. The proliferative responses occur with a concomitant scorbutigenic diet but are inhibited by well-established scurvy.

Mucopolysaccharides from various sources were found to produce mild cardiac lesions, and some produced synovial proliferation as well.

The connective tissue lesions appear to be nonspecific, since they are induced by the injection of a wide variety of substances. Spontaneous streptococcal lymphadenitis was unaccompanied by typical valvular lesions, but induced infections with Klebsiella organisms gave rise to severe valvular and joint lesions.

Histamine, as a possible chemical mediator of antigen-antibody reactions, was administered in lethal doses "covered" by diphenhydramine (Benadryl), but the resulting lesions were of milder form than with many other substances. Trypsin, which produces an anaphylactoid response when given intravenously, was one of the more effective substances studied. Some of the mucopolysaccharides which were not capable of producing hypersensitivity, as judged by the elicitation of anaphylaxis, were capable of producing joint lesions, in contrast to a mucopolysaccharide with antigenic properties. Since the substances giving rise to the severer local response at the site of injection were associated with the more marked cardiac valvular lesions, it is not impossible that such substances may act as proantigens.

There is no direct proof or disproof of hypersensitiveness as the pathogenetic mechanism, but certain aspects suggest that the earliest changes occur before the expected development of tissue sensitivity or circulating antibodies.

While no specific thesis has been proved, it is hoped that these experiments will widen the horizons for future investigations, not only into the field of rheumatic fever but also into that of connective tissue alterations in general.

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JUVENILE MELANOMAS, BENIGN AND MALIGNANT

Fatal Melanoblastoma in a Two-Year-Old Boy

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IN THE last few years it has become increasingly certain that proliferating nevi and related conditions in infants and prepubertal children require clinical and histopathologic evaluation quite different from that appropriate for apparently similar lesions in adults. Prognosis, likewise, presents special difficulties in this group. Even the presence of metastases, while it may provide theoretical confirmation of malignancy, may be an inadequate guide as to prognosis, since several children with metastatic lesions have survived. Under special circumstances, similar survival is noted in adults. Enos and Holmes [1] found that in elderly Negroes in the Canal Zone a primary malignant melanoma of the foot might remain localized, or metastasis might not extend beyond the inguinal lymph nodes. These authors commented on the similarity of these neoplasms in histologic pattern and in clinical behavior to juvenile melanomas.

Fatal malignant melanoblastoma is extremely rare in the prepubertal years. The exact incidence in children is difficult to determine, partly because of lack of adequate criteria for the diagnosis. It is well known that the histologic pattern of the benign juvenile melanoma simulates that of the malignant lesion of adults. Spitz,[2] Allen,[3] and Allen and Spitz [4] have set up criteria for the diagnosis of juvenile melanoma which, they state, are applicable in about two-thirds of the cases.

Because precise criteria have yet to be established, the same difficulties apply to an attempt to survey the literature as to practical diagnosis and prognosis. Examples of malignant melanoblastoma with fatal outcome in children are collected in Table 1. The cases of Dargeon [5] and Weber [7] were instances of transplacental metastases from maternal primary melanoblastoma and therefore fall into a separate, and entirely different, category. The newborn infant presented by Sweet and Connerty [6] had multiple subcutaneous nodules at birth. No primary lesion was described, and the mother showed no evidences of melanoblastoma or any other malignant new growth. The exact classification of the neoplasm in this case may be open to question. The case recently described by Williams [13] resembles that forming the basis of the present report. Malignant proliferation developed in two areas within a very extensive congenital, pigmented, hairy nevus of the upper half of the body. Death occurred at 5 years. There was extensive nodal and pleural involvement, but no metastases were found in the liver.

The ultimate elucidation of the problems of prognosis for patients with juvenile melanomas which have at least some of the morphologic features of malignant melanoblastoma must take into account the considerable group of cases of pre-

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pubertal melanoma with metastases but without a fatal outcome. Perhaps it can be assumed that the prepubertal human body, and particularly the liver, provides a less favorable, or even hostile, environment for blood-borne melanoma cells, in comparison with the body of the adult. Table 2 lists cases of neoplasms resembling malignant melanoblastoma in children, with metastases but without fatal termination.

Numerous authors, de Cholnoky,[18] Traub,[19] Farrell,[20] Daland and Holmes,[21] and Affleck,[22] in reporting large series of melanoblastomas, have included some

TABLE 1.—*Fatal Cases of Prepubertal Melanoblastoma Reported in the Literature*

Author	Age	Sex
Allen and Spitz [4]	12 yr.	F
Allen and Spitz [4]	9 yr.	..
Allen and Spitz [4] *	2 yr.	M
Allen †	5 yr.	M
Dargeon and others [5]	7½ mo.	M
MacDonald [6]	13 yr.	F
Weber and others [7]	8 mo.	M
Sweet and Connerty [8]	Newborn	..
Coe [9]	6 mo.	..
Russo [10]	5 yr.	M
Nowkirischky [11]	11 yr.	F
Truax and Page [12]	8½ yr.	F
Williams [13]	5 yr.	F

* Included by Allen and Spitz [4] by permission. This is the case reported in full in the present paper.
† Knowledge of this case was obtained by personal communication from Dr. A. C. Allen. The case is of special interest because an androgenic adrenocortical carcinoma may have given this patient the equivalent of a postpubertal status. Presumably, a more complete report will be available later.

TABLE 2.—*Nonfatal Cases of Prepubertal Melanoblastoma with Metastases*

Author	Age, Yr.	Sex	Follow-Up Period, Yr.
Allen and Spitz [4]	7	M	..
	10	M	..
Wright [14]	6	..	6
MacDonald [6]	6	M	3
	13	F	1
	14	F	7
Webster and others [15]	8	..	10
	8	F	12
Coffey and Berkeley [16]	7	F	2
Adelson [17]	Under 15	..	5
	Under 15	..	10
Truax and Page [12]	9	F	..

prepubertal cases. With few exceptions, however, they do not give detailed information on specific cases. Pack and colleagues [23] and Sylvén [24] have pointed out that this group must be considered separately in statistical studies, since the results are almost uniformly good. Even further, only five years ago, Pack [25] stated that he had never seen a metastasizing melanoma in a child and that he had doubts concerning those examples which had been reported.

This report includes the clinical and pathologic details of a case of fatal malignant melanoblastoma in the prepubertal age group. The case report is followed by a comparison of the histologic features of this neoplasm, of adult malignant melanoblastoma in general, and of similar lesions in children which have had a benign course as revealed by adequate follow-up records.

REPORT OF A CASE

A male infant, J. D., had had since birth a large saddle-type pigmented, hairy nevus. Even at birth there was a weeping area near the center of the large lesion as viewed dorsally. This denuded area was at waistline level and just to the left of the midline of the back. It apparently healed superficially and developed into a pedunculated dry mass. When the patient was about 2 years old, his mother noted a lump in the left inguinal region. This mass was then 2 to 3 cm. in diameter. This rapidly doubled in size. A biopsy specimen of the inguinal mass was diagnosed (Dr. O. W. Lohr, Saginaw, Mich., and Dr. C. V. Weller, Ann Arbor, Mich.) as malignant melanoblastoma. The site of removal of the biopsy tissue did not heal, became infected, and bled intermittently. The boy was admitted to the University Hospital on April 29, 1949. He was then 2½ years old. There was no family history of neoplasia. The mother had continued in good health.

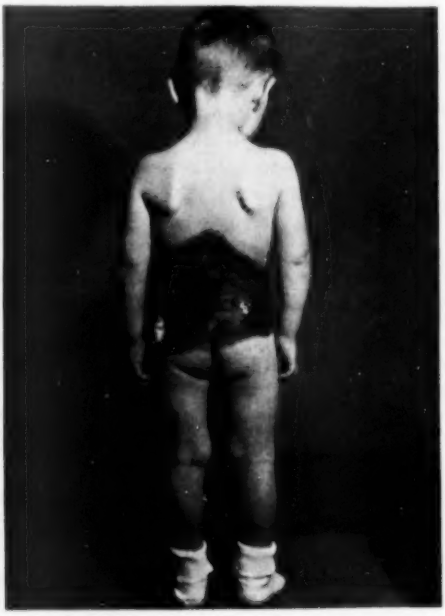

Fig. 1.—J. D., 2½ years old at the time of admission to the University Hospital. A papillomatous structure is evident just to the left of the middorsal line and about at the center of the saddle nevus vertically.

At the time of admission no abnormality of the eyes was noted on ophthalmoscopic examination. There were no abnormal findings related to the lungs or heart. The liver was not palpable. Over the lumbar area there was a large darkly pigmented, hairy nevus with a pedunculated central portion (Fig. 1). In the left inguinal area a lemon-sized hard brown-purple mass was present. The apex of this tumor was ulcerated and fungating. Neurologic examination was negative. No abnormalities of the lungs were seen in roentgenograms taken at admission and one month later.

A week after the patient entered the hospital, the nevus on the back and the left inguinal nodes were excised (Dr. M. S. DeWeese). It was recognized that excision of the inguinal mass had not been complete. The defect on the back was covered by skin grafts. Two weeks later the surgeon attempted to remove the remaining tumor in the inguinal and iliac areas. He found massive involvement of the periaortic lymph nodes and was unable to remove all of the neoplasm. Hepatic metastases were discovered in the course of the abdominal operation. Because the prog-

nosis was considered hopeless, at the parents' request the child was returned to his home for terminal care. The local physician (Dr. J. T. Keyes, Birch Run, Mich.) has reported that the patient grew progressively worse, becoming anorectic and cachectic. Ascites and edema of the left leg developed, and the boy died July 7, 1949. There was no necropsy.

PATHOLOGIC ANATOMY

The first material seen in this department was a prepared slide of tissue from the inguinal node submitted by Dr. Lohr, of Saginaw. There was very little tissue on the slide, and no clinical information was given. There were sheets and indistinct cords and masses of polyhedral cells with neutrophilic cytoplasm and dark pleomorphic nuclei. Large dull-brown pigment particles were moderately numerous. Mitotic figures were not seen. Much of the tissue was necrotic. It was recognized as a poorly differentiated neoplasm, and a tentative diagnosis of melanoblastoma was made, pending the results of special stains to rule out the possibility that the pigment might be hemosiderin. This was done by Dr. Lohr and the diagnosis of melanoblastoma confirmed.

At the first operation at the University Hospital a piece of black skin 16 by 4 by 3 cm., with a central polypoid mass 2 by 2 by 1 cm., was removed (Fig. 2). The flat area was a cellular dermal nevus. No junctional areas were found in numerous large blocks. The central papillomatous mass consisted entirely of large, pale, loosely arranged polyhedral cells. Mitotic figures were numerous. The mass was covered by epidermis, a portion of which was involved in junctional change. This change was not in the form of nests in the epithelium but was more diffuse, with replacement of the basal layer and part of the prickle cell layer by melanoblastoma cells in an obviously junctional pattern (Fig. 3). Pigmentation was moderate. Inguinal and retroperitoneal nodes removed at the first and second operations contained metastatic pigmented melanoblastoma.

COMPARISON OF JUVENILE MELANOMAS AND MALIGNANT MELANOBLASTOMAS IN CHILDREN AND ADULTS

Thirty-five melanotic lesions from children were selected from the departmental files. They were chosen because the original examiners had expressed concern over their cellularity, had compared them with adult melanoblastoma, or, in a few cases, had made a diagnosis of malignant melanoblastoma. For further comparison, nine examples of malignant melanoblastoma from adults were selected. These were all primary lesions which were known to have produced metastases.

Without an attempt at semiquantitative grading methods, it was apparent that the lesions from children could be arranged in order from obviously benign cellular compound nevi to those having the histologic attributes of malignant melanoblastoma. At the well-differentiated extreme of the series, the junctional component was slight, with small nests of a few cells each in the epidermis. These nests were made up of small darkly staining cells, arranged compactly. As the members of the series approached the histologic pattern of malignant melanoblastoma, the cells in the junctional nests were large, had hyperchromatic nuclei, and were separated from one another within the nests. Within some of these loosely arranged nests were large cells with one or several very deeply staining nuclei. When these nests were completely enclosed in epithelium, although the wall of squamous cells might be very thin, the histologic picture was that of the active junctional nevus of the

adult. In the juvenile melanomas the pattern differed in that the junctional islands were not confined by epidermal cells and invaded the dermis to a slight degree. Mitotic figures were present but were not numerous (Fig. 4). The prepubertal malignant melanoblastoma which is described in detail in this paper differed from

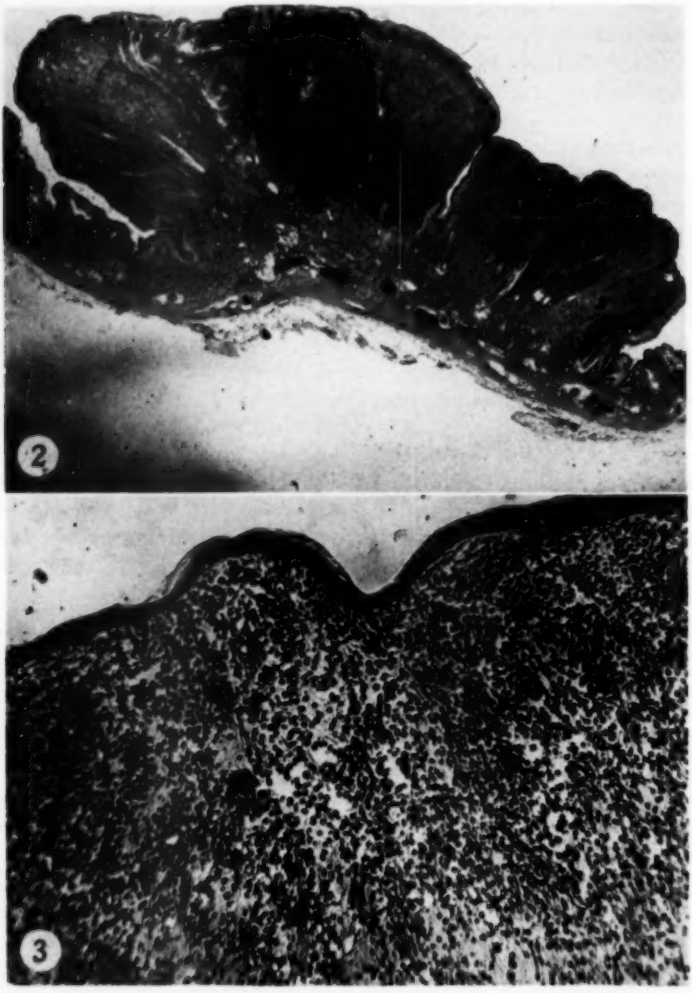

Fig. 2.—A low-power view of the papillomatous structure seen in Figure 1 after excision (8402-BA). Hemalum and eosin; × 5.

Fig. 3.—High-power view of a part of Figure 2. It is evident that there is diffuse and continuous junctional involvement of the epidermis. There is no basal layer in this area. Hemalum and eosin; × 100.

the usual juvenile melanoma in that the junctional component was not confined to isolated nests but constituted a continuous zone involving the lower levels of the epidermis.

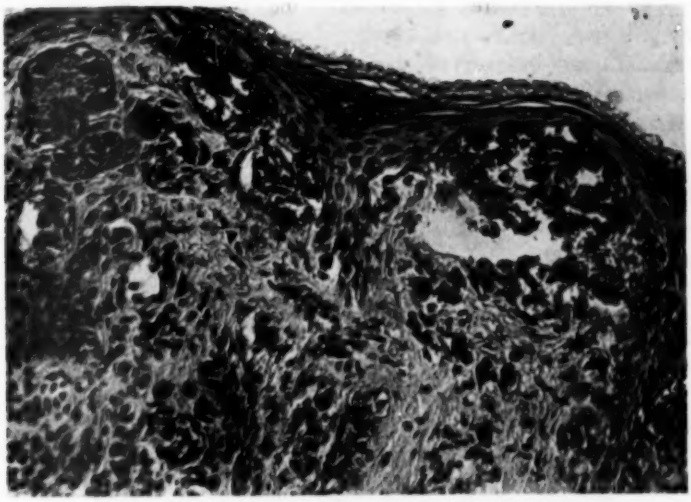

Fig. 4.—Typical juvenile melanoma from another case (931-LBE). The junctional areas are in discrete nests, although they are not confined to the epidermis. Hemalum and eosin; × 100.

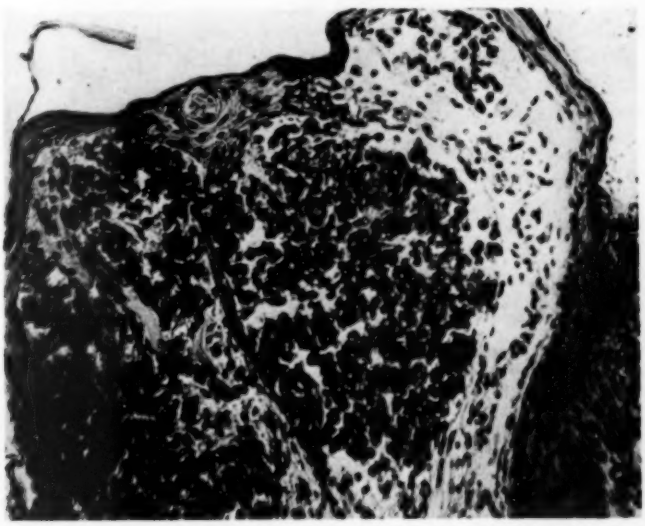

Fig. 5.—A lesion from an 18-month-old child (9599-LBE) with diffuse junctional change largely replacing the lower layers of the epidermis. This section may be compared with Figures 3 and 6. In spite of the resemblance of this tumor to malignant melanoblastoma, this child is living and well after three and a third years. Hemalum and eosin; × 100.

In the group of 35 juvenile lesions from the diagnostic files of this department, 8 were found which contained the same histologic features as the fatal case. One other lesion lacked the epidermal changes, but the neoplastic cells in the dermis could not be distinguished from those of the fatal case. However, this latter example had been removed from a 15-year-old girl, who died with extensive metastases three years after its excision. No menstrual history was available, but it is probable that this patient had passed the menarche. Additional information was obtained for five of the eight cases that were so similar to the known malignant example. All five

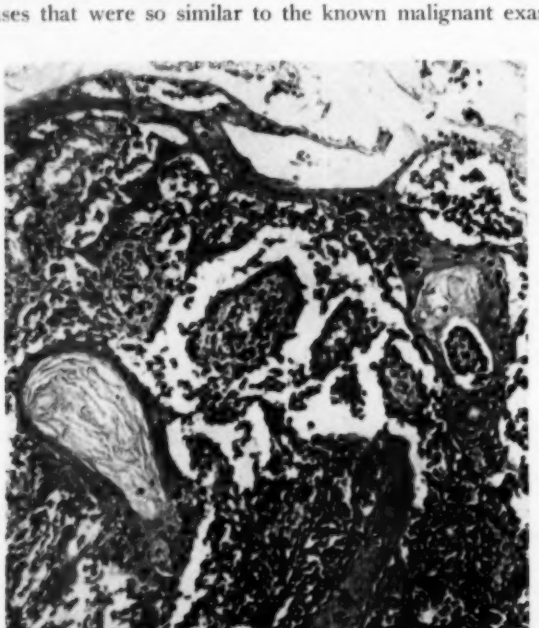

Fig. 6.—A primary malignant melanoblastoma from an adult (2105-BC) who subsequently had metastases. Diffuse junctional areas, similar to those in Figures 3 and 5, are present here also. Hemalum and eosin; × 100.

patients were reported to be living, without evidence of neoplasm, after intervals ranging from 20 months to 10 years. None had received additional treatment.

Comparison of the malignant lesion of the child with the nine primary malignant neoplasms of adults demonstrated a striking similarity. Particularly noteworthy in this group was the distribution of the junctional components in broad zones rather than as discrete nests (Fig. 5).

The fruitful comparisons in this study have all been based on junctional activity alone. No correlation between extent of benign dermal involvement or the amount of pigment and the degree or character of junctional change was apparent.

COMMENT

The present case is of interest as a fully substantiated example of a juvenile malignant melanoblastoma. As such, it presents an extremely rare condition and, in addition, gives an opportunity to compare a malignant melanoblastoma in a child with similar, but benign, juvenile melanomas and with malignant melanoblastomas of the adult.

It is possible to arrange nevi, nonmalignant juvenile melanomas, and both juvenile and adult malignant melanoblastomas (Fig. 6) in series varying in respect to increasing degrees of cellularity, lack of cellular cohesion, size of cells, lateral extent of the junctional change, and depth of dermal invasion. The variants grade almost imperceptibly into one another, and in prepubertal children there is a group which appears to be malignant by all present histologic criteria but which, in the great majority of cases, is clinically benign. In the wide range of variation of both the benign and the malignant melanomas are found examples which meet perfectly the criteria of Spitz [2] and of Allen and Spitz [4] for juvenile melanomas, but precise compartmental assignment, although it would be invaluable, appears to be impossible. The differences between benign juvenile melanomas and adult malignant melanoblastomas appear to be quantitative rather than qualitative. Within the childhood group the differences between clinically benign melanoblastomas and histologically similar but clinically malignant melanoblastomas are, as yet, not understood. There is increasing evidence that the answer may be found in the steroid complex of the host.

The melanomas which are histologically malignant can be differentiated from juvenile melanomas in general by the spreading of the junctional reaction so that the nevus cells no longer appear as discrete nests, but cover a wide area in which the lower layers of the epidermis are replaced. It is tempting to consider the present study series as illustrative of the histogenesis of malignant melanoblastoma in junctional nevi. While this may be true, more conclusive evidence is needed.

SUMMARY

A case of fatal malignant melanoblastoma in a 2-year-old boy is presented in detail.

The lesion in this case is compared with other melanomas of children which were either histologically benign or histologically malignant and clinically benign. A continuous series of increasing junctional activity can be demonstrated in the group as a whole.

The neoplasm in the fatal case in the child was histologically similar to adult primary malignant melanoblastomas.

In adult and prepubertal malignant melanoblastomas and in those prepubertal melanomas with malignant appearance but benign course the junctional component is not in nests but is diffuse, replacing the lower levels of the epidermis over wide areas.

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RECENT DEVELOPMENTS IN ENVIRONMENTAL CANCER

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(Concluded from page 523)

H. Specific Dietary Factors

1. *Iodine.*—The excessive incidence of carcinoma of the thyroid in regions with endemic goiter due to iodine deficiency in the soil, drinking water, and foodstuffs represents another example of an environmental cancer in man on the basis of dietary imbalances.[928] These cancers originate from nodular adenomatous goiters, especially the mononodular ones.† Since it is not likely that iodine deficiency as such provides a specific carcinogenic stimulus to thyroid tissue, it is possible that such an action is related to an imbalance of the pituitary hormones associated with disturbances of the thyroxine production. It may be mentioned that Bielschowsky [947] found in rats suffering from chronic thyroxine deficiency due to an inadequate iodine content of the diet, basophile pituitary adenomas and thyroid adenomas.

2. *Thiourea and Thiouracil.*—This pituitary concept receives some support from the observation of adenomas and carcinomas of the thyroid ‡ in rats and, possibly, man fed various thiourea and thiouracil derivatives and of hepatomas in rats given orally thiourea and thioacetamide.§ These chemicals are used for various medicinal purposes (antithyroid drugs, exophthalmic goiter, coronary sclerosis, chronic ulcerative colitis [936]), as preventives of orange decay, and for the fattening of meat animals. Thiourea derivatives are extensively used as accelerators in the rubber industry. Because of these tumorigenic effects of thiourea and its derivatives, the use of these substances for the preservation of citrus fruit has been prohibited in this country. Another experimental counterpart are the thyroid adenomas found in rats fed rape seed containing antithyroid chemicals apparently related to thiocyanates. It is not believed that these antithyroid drugs are the primary factors in the carcinogenic processes, but it is thought that they create mainly the proliferative soil for an enhanced action of specific carcinogens, such as 2-acetylaminofluorene.[937]

3. *Fluorine.*—The close chemical relations and the possible biologic antagonism between iodine and fluorine, in addition to the widespread exposure to the latter chemical for industrial or dietary reasons (naturally or artificially fluoridated drinking water), provide an indication for briefly commenting on the data connecting exposure to fluorine with neoplastic manifestations. There is no evidence that the severe chronic dental, bony, and joint disturbances associated with chronic indus-

National Cancer Institute, National Institutes of Health, Public Health Service, and Department of Health, Education and Welfare.

† References 929-935.

‡ References 937-944.

§ References 945-947.

trial or endemic fluorosis have given rise to neoplastic growths from such tissues.|| The question, however, has been raised whether the ingestion of fluoridated drinking water might be responsible for the development of goiter ¶ because of the special affinity of fluorine for the thyroid.

4. *Selenium.*—Excessive amounts of selenium in the soil and in foodstuffs have been reported from regions in the United States (Wyoming, South Dakota) and have given rise there to chronic selenosis ("alkali disease") among livestock,# characterized by cirrhosis of the liver and glomerulonephritis. There occurs, moreover, extensive contact in industry with various selenium compounds.[957] Selenium sulfide has recently been introduced as a medicinal agent for external application in certain skin infections.* Inhabitants of regions with seleniferous soil showed, among other symptoms, a frequent mild to intense icterus, suggesting liver damage.† There is no evidence that selenotic liver injury has given rise to primary cancer of the liver in this country or in South Africa (Berman), although little is known as to the selenium content of African soil. Recent experimental studies of Nelson, Fitzhugh, and Calvery [963] on rats fed seleniferous grain resulted in the development of liver cirrhosis complicated by adenomatoid hepatic nodules and low-grade hepatocarcinomas in animals surviving this dietary regimen for more than 18 months. It appears from this observation that the chronic hepatotoxic effects associated with selenosis on a dietary or an occupational basis may represent a precursor to hepatic carcinoma and that epidemiologic studies of exposed human and animal population groups on this account seem to be in order.

5. *Dulcin (p-Phenetylurea).*—The sweetening agent dulcin, which for many years was commercially available, was recently found to elicit cirrhosis and tumors of the liver in rats given this chemical by mouth,[967] necessitating its withdrawal from the open market.

6. *Chlorinated Hydrocarbons.*—The large scale and increasing use of chlorinated hydrocarbons as pesticides (insecticides, herbicides) and industrial solvents has brought large parts of the general and industrial population into close contact with a group of chemicals displaying often marked hepatotoxic properties. The possibility of a slow and insidious injury upon prolonged low-level accumulative exposure exists, because these substances seem to be retained over long periods of time in the fat tissue. Toxic contact with these chemicals may be not only direct (occupational exposures, domestic use of chlorinated hydrocarbons containing sprays and dusts, consumption of foodstuffs with insecticide and herbicide residues, degreasing agents, dry cleaning agents, ingredients of paints, paint removers, liquid waxes, etc.), but also indirect, i. e., by the ingestion of milk or fat from animals which absorbed these chemicals into their system for various reasons.‡

Severe and acute hepatic degeneration was observed in man after ingestion of D.D.T. (chlorophenothane),[974] and after acute occupational exposure to carbon tetrachloride,§ while cirrhosis of the liver after prolonged contact with this chemical

|| References 948-952 and 955.
¶ References 953 and 954.
# References 956-962 and 966.
* References 963 and 964.
† References 958-962.
‡ References 968-972 and 975-982.
§ References 983-987.

was recorded by Poindexter and Greene,[988] with tetrachlorethane and tetrachloroethylene by Willcox and Spillsbury,[989] and Coler and Rossmiller,[990] with trichloroethane by Fiessinger and co-workers,[991] with methyl chloride by Wood,[992] and with pentachlorinated naphthalenes by Cotter.[993] There is no evidence that exposures to these chlorinated hydrocarbons have produced benign or malignant tumors of the liver in man.

Several observations made during recent years in mice and rats, on the other hand, attest the fact that such neoplastic manifestations can be produced by prolonged feeding of carbon tetrachloride, chloroform, or chlorophenothane (D. D. T.). Benign and malignant hepatomas in mice followed upon an oral administration of carbon tetrachloride || and of chloroform.[1001]. The occurrence of nodular adenomatoid hyperplasias and hepatomas in rats fed large amounts of D. D. T. suggested to Fitzhugh and Nelson [1002] that D. D. T. possesses a distinct, although minimal, tumorigenic tendency.

Although it does not appear very likely that there exist any opportunities for a prolonged and effectively toxic exposure of man to D. D. T. sufficient for eliciting, by itself, hepatomatous or hepatocarcinomatous reactions, it is conceivable that such exposures may play a contributory hepatotoxic and hepatocarcinogenic role in conjunction with other environmental hepatotoxins, such as arsenic, chlorinated aliphatic and aromatic hydrocarbons, and selenium, and with hyperthyroidism,[1003] starvation, alcoholism, and infectious agents. In assessing degrees of environmental carcinogenic hazards for individual agents, it is important to keep in mind the frequent coexposures to syncarcinogenic agents, as well as the fact that the general population upon which such agents may act is composed not only of healthy persons, but also of sick ones having an impaired resistance and metabolism.

7. Spices and Alkaloids.—In commenting on the possible role which the consumption of strongly spiced food might have on the development and high incidence of primary cancer of the liver, Berman ¶ pointed out that it was unlikely that any relation between these two factors could exist, because the food of the Bantus, who have a high incidence of liver cancer, is little spiced, while that of the Javanese, also suffering from an excessive liver cancer liability, is highly spiced, as is the food of the Asiatic Indians, who have a much lower liver cancer incidence. In support of his opinion, he cited the negative results which Hieger reported in 1940, when he fed rats and mice a diet containing large amounts of ground Java chilies.

However, the recent discovery that several such vegetable agents when given orally elicit cancers of the liver in rats has definitely reopened this question. Hoch-Ligeti # succeeded in producing cirrhosis, hepatomas, multiple cystic cholangiomas, solid adenomas, and adenocarcinomas in rats given a basic diet with the addition of chilies, which were obtained from Java and belonged to the genus Capsicum, of the family Solanaceae, and were used as a spice in Africa and Asia. These chilies contain a pungent substance (capsaicin), a red coloring matter, capsanthin, and a fixed oil.

In 1920 Willmot and Robertson [1006] reported the occurrence of hematemesis, melena, enlargement of the liver, and ascites among poorer classes of Europeans

|| References 994-1000.

¶ Reference 30.

References 1004 and 1005.

living in South Africa and attributed this syndrome to poisoning with the weeds Senecio ilicifolius and Senecio burchelli, containing the alkaloids retrorsine and isatidine, because similar symptom complexes have been found among cattle following the ingestion of this weed. In view of the medicinal use of Senecio plants against nightmares in children, and as a stimulant, cold remedy, etc., by different African tribes having a high primary liver cancer incidence, Cook, Duffy, and Schoental [1007] fed the alkaloids of Senecio Jacobæa to rats and obtained hepatomas. The two Senecio alkaloids, which chemically are similar to capsaicin obtained from chilies,[1007] produce cirrhosis of the liver and hepatomas in rats. The investigators concluded from these observations that the prevalence of liver tumors among populations known to use concoctions of Senecio plants from childhood suggested that this practice might play a part in the etiology of primary liver cancers.

Since crude ergot may contaminate rye bread, while ergot preparations have found a wide medicinal use, it may briefly be mentioned that rats fed ergot developed after two years neurofibromatous nodules of the ears.[1009] There do not exist any corresponding observations in man following a prolonged dietary or medicinal intake of ergot, although epidemics of ergot poisoning have at times affected larger bodies of population.

Mention may also be made in this connection of the epidemiologic evidence suggesting that vegetable extractives of tobacco may be responsible for the prevalence of cancer of the oral cavity among persons chewing tobacco or quids (betel quid, khaini quid)[1010] containing tobacco as an ingredient.[1011] Schoental * considered the possibility that contact with tobacco alkaloids inhaled while smoking tobacco may be related to cancers of the lung and buccal cavity. Applications of commercial nicotine and its oxidation products applied to the mouth and skin of mice, however, have so far given negative results.

Recent observations on an excessive frequency of lung cancer among Indonesian workers employed in tobacco plantations † have provided new substance to the previous epidemiologic evidence suggesting such an excessive liability for German tobacco workers and cigarette manufacturers [1022] exposed to the inhalation of tobacco dust. There exists a complete lack of information on this aspect from American sources despite the large number of persons engaged in the cultivation, storing, processing, transporting, and handling of tobacco and in the manufacture of smoking tobacco, cigars, cigarettes, snuff, and chewing tobacco and the use of powdered waste tobacco as a fertilizer and for the production of nicotine. A comprehensive investigation of these population groups appears to be indicated in view of the observation of a highly excessive lung cancer mortality among a relatively small number of cigar manufacturers during the course of a state-wide environmental cancer survey.

8. *Diethylene Glycol.*—Ingestion and inhalation of diethylene glycol may occur when smoking cigarettes processed with diethylene glycol as a humectant. When this chemical was fed to rats over many months, stones, fibropapillomas, and one carcinoma were found in the bladders of some of these animals.[1014] Because of the relatively large amounts of diethylene glycol administered (1% in diet), it seems very doubtful whether these observations possess practical significance as to the

* References 1012 and 1013.

† References 1020 and 1021.

existence of a human hazard from such a source, although they may for rubber manufacturers having cutaneous and respiratory contact with this chemical.

9. *Tannic Acid.*—Tannic acid, a constituent of many foodstuffs (fruits, coffee, tea, wine), was recently shown to produce cirrhosis of the liver, hepatomas, and cholangiomas in rats given this chemical by repeated parenteral injections.‡ Some of these tumors revealed an atypical cellular pattern and invasion of hepatic veins suggesting low-grade malignant process. There were, moreover, in some of the animals bronchial adenomas. Tannic acid applied to ulcerated surfaces of the skin of rats, and imitating its medicinal use in the treatment of burns, did not cause cirrhosis or tumors of the liver. When given orally, tannic acid in appropriate dosage, on the other hand, produced hepatotoxic reactions in rats. It is uncertain whether tannic acid is a specific carcinogenic agent or whether the liver tumors are merely the result of hepatic damage caused by tannic acid. Although the present evidence does not indicate the existence of direct relations to liver cancers in man, it provides, nevertheless, additional support to the concept that environmental, hepatotoxic and cirrhosis-producing agents may exert a direct or an indirect causal influence upon the genesis of liver tumors.§

10. *Heated Fat.*—The socioeconomic and topographical distribution of cancers of the alimentary tract provides circumstantial evidence suggesting the action of environmental and, especially, dietary causal factors. In view of the carcinogenicity of methylcholanthrene, the suspicion arose that other derivatives of cholesterol, especially those formed during the repeated and excessive heating of cholesterol during the preparation of food, might be involved in the etiology of gastric cancer.‖ It was found that heating of fat carried out at temperatures above 325 C. in an iron frying pan results in a pyrolysis of fat.¶ There occurs under such conditions a ketonization and polymerization of the glycerides,# formation of acrolein,[1025] and, possibly, also aldehydes.*

The feeding of cholesterol heated to 300 C. to rats for periods up to two years led to an induced avitaminosis A associated with papillomatosis of the forestomach, but not to hyperplastic or neoplastic reactions of the glandular stomach.† Feeding of superheated fat to rats and mice was followed by the development of papillomas and squamous cell carcinomas of the stomach.‡ Roffo § reported also the occurrence of malignant tumors of the glandular stomach in rats given orally fat preheated to 350 C. Lane, Blickenstaff, and Ivy,[1029] who repeated this experiment, however, obtained only papillomas of the forestomach and ulcers of the glandular stomach. In a few rats injected subcutaneously with heated lard or vegetable oil, sarcomas at the site of injection were noted.[1038]

In view of the failure to produce malignant lesions of the glandular stomach consistently, if at all, with superheated fats and cholesterol, it is somewhat doubtful

‡ References 1015-1019.
§ References 1015-1018.
‖ References 1023-1025.
¶ References 1026 and 1027.
# References 1028-1030.
* References 1025 and 1031.
† References 1023, 1025, and 1032.
‡ References 1026, 1027, and 1033-1038.
§ References 1033-1036.

whether these observations have any significance for the human gastric cancer problem. They suggest, nevertheless, the possibility that a variety of chemical substances formed by the heating of carbonaceous matter used as food may be involved in the production of cancers of the alimentary tract. Consideration in this respect should be given to the formation of carcinogenic polycyclic hydrocarbons generated by the heat cracking of carbonaceous material, of oxidation products of cholesterol, of aliphatic acid polymers, and of epoxides.

11. *Carbamates.*—Carbamates, particularly ethyl carbamate (urethan), were used for years in medicine as hypnotics, and have been used more recently as antileukemics agents.‖ During the past few years several carbamate esters, especially isopropyl-n-phenylcarbamate and its chlorinated derivatives, have been introduced as weed killers.¶

Urethan, having a pronounced mitosis-inhibiting action, has occasionally produced, when given in large doses to leukemic patients, fatal agranulocytosis.[1040] In agreement with other anticancerous therapeutic agents, urethan also has been found to be carcinogenic.# Nettleship and Henshaw first reported the induction of pulmonary adenomas in mice treated with urethan.[1048] These observations have been confirmed in mice * by others, who noted also the presence of malignant pulmonary tumors, and in rats.† Other carbamate derivatives were found to be either very much less effective in tumorigenic respects or totally ineffective (isopropyl-n-phenylcarbamate).[1048] There does not exist, therefore, at present any experimental evidence indicating that an accidental ingestion of contaminated vegetation or an occupational exposure to this weed killer may be associated with a cancer hazard.

I. Miscellaneous Potential Carcinogens

1. *Mustards.*—The induction of tumors in mice and rats by the administration of several of the nitrogen and sulfur mustards used as anticancerous chemicals in man provides another demonstration of the ambivalent properties of many of the growth-inhibiting agents.‡ Clinical usage of these agents should be viewed with concern as a consequence of these observations.

2. *Beryllium.*—Beryllium and beryllium compounds present another example of a potential environmental carcinogen. Of the approximately 500 cases of chronic berylliosis reported in the United States since 1943, by far the most have occurred in processing of the metal from ores and are due to the inhalation of finely divided beryllium.[1059] About 100 cases were recorded among employees of the fluorescent lamp industry, while the remaining cases were traced to the manufacture of fluorescent powder, machining of metallic beryllium, production of beryllium alloys, sign-tube manufacture, ceramic production, crystal manufacture, and laboratory work with beryllium and its compounds.§ Neighborhood cases were observed among persons living in the vicinity of fluorescent lamp salvage operations and fluorescent lamp manufacturers.[1061] Granulomatous, "sarcoid" lesions developed in the lung after

‖ References 1040-1043.
¶ References 1044 and 1045.
# References 1046 and 1047.
* References 1049-1051.
† References 1052 and 1053.
‡ References 1046, 1047, and 1054-1058.
§ References 1060, 1062-1064.

the inhalation of beryllium and its compounds and in the subcutaneous tissue when beryllium enters through cuts. Beryllium is probably deposited in the greatest quantity in the reticuloendothelial tissues of the lung, from which Be ions are released to form a Be-protein complex which acquires antigenic properties.[1060] The light metal beryllium thus seems to react with the tissue proteins in a fashion similar to that noted for other carcinogenic metals (chromium, nickel, arsenic). Larger amounts of beryllium are finally stored in the bones.[1065]

These reactions to beryllium and its compounds are of importance as potential cancer hazards, for several investigators have succeeded in producing in rabbits osteogenic sarcomas appearing from 11 to 24 months after repeated intravenous injections of various insoluble beryllium compounds || or after prolonged inhalation of beryllium oxide.¶ The development of bronchogenic carcinomas in rats which inhaled, for over 18 months, dust of soluble and insoluble beryllium compounds was recently reported by Vorwald.[1072]

Opinion as to the human significance of these findings is divided. While some observers maintain that occupational, environmental, or accidental contact with beryllium compounds does not entail a potential cancer hazard, others feel that a hazard may exist.# Persons surviving sufficiently long a berylliotic condition should be watched, at any rate, for late malignant sequelae. Likewise, persons affected by cancer of the lungs or bones should be questioned about previous exposures to beryllium and its compounds. Three cases of lung cancer and berylliosis have been reported.

3. *Macromolecular and Polymerized Chemicals.*—Recent experimental observations on the carcinogenic action of a number of synthetic polymers when implanted into mice and/or rats have become of considerable scientific and practical significance, for two reasons: 1. An entirely new field of environmental substances has been opened for investigation.* Many of the synthetic plastics are used as wrapping material of foodstuffs; as containers of drugs, cosmetics, and sanitary goods; as textile fibers in wearing apparel, where they have direct contact with the human skin; as medical prostheses (dentures, intraocular lenses, surgical films and tubes embedded into tissues, substitutes for tympanic membranes, joint surfaces and skull bones, sutures, and fibrous tissue stimulants),† and as ion-exchange agents taken internally.‡ 2. They may provide an important clue to a better understanding of the carcinostatic action of certain chemicals and of the ambivalent action of some carcinogens.§ Model experiments on the action mechanism of aromatic nitrogen mustards have shown that they react readily with the ionized carboxyl groups of polymeric systems, e. g., polymethacrylic acid, and that the degree of this affinity increases with the degree of polymerization. These results are of considerable interest in connection with the study of reactions of carcinostatic and carcinogenic mustards with nucleic acids and with biologic material generally.[1090] It has been found, moreover, that certain polymethylene compounds without or with replacement of

|| References 1065-1070.
¶ References 1071 and 1072.
# References 1071, 1072, and 1075.
* References 1096 and 1097.
† References 1076-1087.
‡ References 1088 and 1089.
§ References 1096 and 1097.

one or more carbon atoms by nitrogen, sulfur, or oxygen inhibit tumor growth,‖ possibly by multipoint attachment to nucleoproteins or proteins shielding or inhibiting the activity of essential active sites on these molecules.[1092] Mention may be made in this connection of the hypothesis of Hendry, Rose, and Walpole [1093] that the carcinostatic action of some of the methylolamides, epoxides, and ethyleneimines might be related to an intracellular formation of a small polymer, because such compounds are characterized by the ease with which they polymerize. One of these compounds also has displayed carcinogenic properties.

It may perhaps be important to note that macromolecules, such as polymethacrylic acid, polystyrene, and polyvinyl chloride, are degraded by ionizing radiations (x-rays, gamma rays) in the presence of oxygen [1094] and by ultrasonics.[1095]

The macromolecular plastics which have been shown to elicit sarcomatous reactions in rats and/or mice are Cellophane, polyethylene, polyvinyl chloride, polymerized tetrafluoroethylene (Teflon), polysilicone (Silastic), polyamides (hexamethylenediamineadipate-Nylon and ε-caprolactam), polyacrylate, polystyrene, and formaldehyde-phenol plastic (Bakelite).¶ In an attempt to explain the carcinogenic action of these polymers, Druckrey suggested that they may become bound by cross linkage to cellular proteins or that low-grade polymer constituents of such plastics may become incorporated into protein molecules, thereby giving rise to abnormal proteins, which, in turn, would be biologic equivalents to the carcinogen-protein complexes formed by carcinogenic polycyclic hydrocarbons (3,4-benzpyrene) and metals (arsenic, chromium, nickel).

The significance of these observations for the environmental cancer problem is at present still uncertain. It is obviously unwise to continue the practice of implanting into human beings, for medical reasons, polymers which have shown carcinogenic properties in animals until we are certain that these macromolecular substances are not carcinogenic to man. From the evidence on hand, it does not appear to be likely that even a prolonged external contact with these chemicals is associated with any cancer hazard. However, again, it may be desirable to study particularly population groups having intimate occupational exposures to these chemicals for any abnormalities in cancer incidence and cancer distribution by sites.

The claim was recently advanced [1076] that dental prostheses made of methylmethacrylate plastics increase the liability of the wearers to cancer of the mouth, thereby chemospecifically delineating former allegations concerning a causal connection between the wearing of dentures and intraoral cancer.#

Since asbestos is a silicon polymer, there exists the possibility that the cancers of the lung associated with pulmonary asbestosis have a polymer background and thus may belong to the group of "polymer cancers." Similar considerations may be applied to the causation of the cancers of the nasal sinuses, larynx, and lung seen among isopropanol workers, because the crude liquor to the vapors of which these workers became exposed contains polypropylenes and epoxides [1063] (Fig. 4).

The absence of any carcinogenic complications in the many persons who received intravenous injections of various plasma substitutes composed of dextrose polymers and polyvinyl compounds is reassuring. But in view of the relatively short span of

‖ References 1090 and 1091.
¶ References 1098-1107.
# References 1076 and 1108.

time elapsed since their introduction of these substitutes, there is no definite guarantee as to their absolute lack of carcinogenic properties. Such potential sequelae deserve due consideration, since man and experimental animals to which these and similar plasma substitutes were administered showed marked proliferations of the reticuloendothelial cells incited by the intracellular retention of these macromolecular polymers.[1109]

4. *Tobacco-Smoking Habit.*—(*a*) Epidemiologic Data: Rather far-reaching, if not extravagant, claims recently have been advanced as to the important, if not predominant, role which cigarette smoking is alleged to have played in the production of lung cancer and its progressive rise in frequency during the past 50 years. A critical and sober analysis of the evidence offered in support of these assertions is in

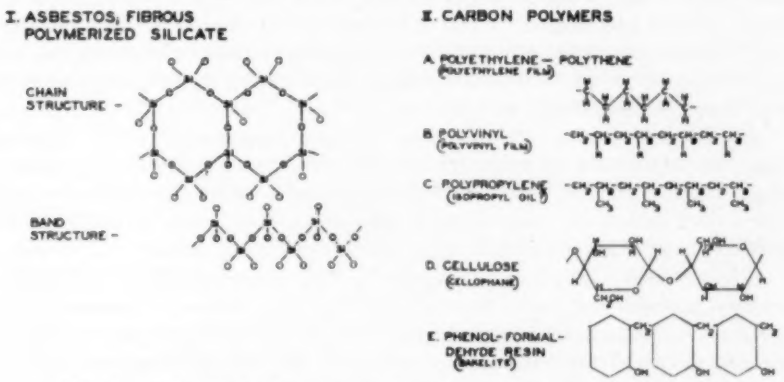

Fig. 4.—Chain and band structures of carcinogenic polymerized chemicals.

TABLE 21.—*Degree of Tobacco Consumption Among Eighty-Six Lung Cancer Cases and Eighty-Six Normal Controls* *

Degree of Tobacco Consumption	Highly Excessive		Very Heavy		Heavy		Moderate		Non-smokers	
	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases
Percentage of degrees among lung cancer series.....	29	25	21	18	15	13	31	27	4	3
Percentage of degrees among normal controls.....	5	4	6	5	25	22	48	41	16	14

* From Müller (Müller, F. H.: Ztschr. Krebsforch. **49**: 57, 1939, published by Springer-Verlag, Berlin).

order not only for reasons of scientific accuracy, but also for medicolegal reasons, and especially for determining the direction of future epidemiologic research and of control activities in the field of lung cancer.

Müller [1110] did the first statistical study on the relation of tobacco smoking to lung cancer by comparing the relative intensity of the smoking habit (cigarettes, cigars, pipe) among the members of a series of 86 lung cancer patients with the intensity distribution among a normal control group (Table 21).

According to the occupational data given, there were in the cancer series 19 male patients occupationally exposed to metal dusts and fumes, lubricating oil mist, and soot; 12 exposed to soot and automobile exhaust; 11 exposed to ingredients of paints,

and 1 exposed to chromates, while of the 10 female cancer cases, 3 had worked in an ammunition plant and 1 in a cigarette factory. A possibly significant occupational exposure history thus existed in 43 of the 76 male cases and in perhaps 4 of the 10 female cases.

An analysis of the tobacco-smoking history of 93 lung cancer patients of Schairer and Schöniger [1111] revealed similar statistical correlations, since 29 were highly excessive smokers, 19 very heavy smokers, 31 heavy smokers, and 11 moderate smokers, while 3 were nonsmokers.

While these discussions of a possible causal relation between cigarette smoking and lung cancer first aroused little attention beyond the narrow circle of research workers, the problem started to attract wide attention from the medical profession, public press, industry, and laity after the publication, in 1950, of the papers of Schrek, Baker, Ballard, and Dolgoff [1112] and of Wynder and Graham.[1113] There followed in rapid succession a number of statistical investigations of this problem from this country and abroad.* From the results of some of these studies, the following conclusions were drawn by the different investigators:

Wynder and Graham [1113]: Excessive and prolonged use of tobacco, especially cigarettes, seems to be an important factor in the indication of bronchogenic carcinoma. Among 605 men with bronchogenic carcinoma, other than adenocarcinoma, 96.5% were moderately heavy to chain smokers for many years, as compared with 73.7% among the general male hospital population without cancer.

Schrek, Baker, Ballard, and Dolgoff [1112]: The correlation between smoking and cancer is probably not due to fortuitous or secondary factors. It seems plausible, therefore, to formulate the hypothesis that there is a direct relationship between cigarette smoking and cancer of the respiratory tract and that cigarette smoking may be a carcinogenic agent. The relatively low percentage of deaths by cancer of the respiratory tract as compared with the high percentage of smokers indicates that smoking is, at most, only a weak carcinogenic agent.

Ochsner, DeCamp, DeBakey, and Ray [1116]: There is a distinct parallelism between the sale of cigarettes and the incidence of bronchogenic carcinoma. Because the carcinogenic effect of cigarette smoking does not become evident until after many years of smoking (approximately 20), it is frightening to speculate on the possible number of bronchogenic cancers that may develop as the result of the tremendous numbers of cigarettes consumed in the two decades from 1930 to 1950. If there is a causal relationship between cigarette smoking and bronchogenic carcinoma, the deaths per 100,000 population from this cause may be expected to increase from 11.3 to 29.4 by 1970.

Levin, Goldstein, and Gerhardt [1114]: These data support the conclusion that lung cancer occurs approximately 65% more frequently among males who have smoked cigarettes for 25 years or more than among males who have smoked cigars or pipes for a comparable period, or among nonsmokers. The data indicate also that pipe and cigar smokers have no higher incidence of lung cancer than nonsmokers. The findings suggest, although they do not establish, a causal relation between cigarette and pipe smoking and, respectively, lung and lip cancer.

Mills and Porter [1125]: Among cancers of the respiratory tract from the larynx downward, an abnormally high percentage of cigarette smokers, as well as of pipe

* References 1114-1139.

and/or cigar users, is found. This group of cancer victims exhibits significantly increased percentages in all forms of smoking.

Doll and Hill †: Among the smokers, a relatively high proportion of the patients with carcinoma of the lung fell in the heavier-smoking categories. Smoking is a factor, and an important factor, in the production of carcinoma of the lung. The risk of developing carcinoma of the lung increases steadily as the amount of cigarette smoking increases. If the risk among nonsmokers is taken as unity and the resulting ratios in the three age groups in which a large number of patients were interviewed (ages 45 to 74) are averaged, the relative risks become 6, 19, 26, 49, and 65 when the number of cigarettes smoked a day are 3, 10, 20, 35, and, say, 60—that is, the midpoints of each smoking group. Cigarette smoking was more closely related to carcinoma of the lung than pipe smoking. No distinct association was found with inhaling.

It appears from the speculations of Doll and Hill that among the population of Greater London over the age of 45, those who smoke 35 or more cigarettes a day had a chance of developing cancer of the lung which was 50 times as great as that of

TABLE 22.—*Statistical Correlations Between Tobacco Smoking and Lung Cancer; Degree of Smoking Habit Among Lung Cancer Patients (Males)*

Authors	Highly Excessive	Very Heavy	Heavy	Moderate	Nonsmokers
Schrek and co-workers	18.3	50.0		12.2	14.6*
Wynder and Graham	20.3	30.9	35.2	12.4	1.3
Doll and Hill	5.0	21.0	30.3	38.6	5.1
Breslow	15.3	50.7	19.5	3.5	9.0
Gsell	30.0	37.0	21.0	10.5	2.0
Controls					
Wynder and Graham	7.6	11.5	36.5	30.5	14.6
Doll and Hill	2.1	11.4	30.5	47.1	8.8
Breslow	3.5	34.8	18.8	11.1	30.5

* Cigarettes only; rest, pipe and cigars.

nonsmokers of similar age. Assuming that these conclusions are essentially correct, it may then justly be argued that an effective control of cigarette smoking offers the means for a far-reaching prevention of cancer of the lung and, possibly, the larynx. The statistical data which form the basis of these conclusions are summarized in Table 22. Brunner found among 127 lung cancer patients, 75% heavy smokers and 9.5% nonsmokers.[1132]

Sadowsky, Gilliam, and Cornfield [1139] concluded from their analysis that the magnitude of the relative risk of lung cancer among cigarette smokers was about five times that in nonsmokers, and that there was a real statistical association between cigarette smoking and laryngeal cancer and between pipe smoking and cancer of the lip. They leave open the question whether or not smoking is etiologically related to lung cancer.

An even less positive note was recently furnished by the investigations of Denk,[1140] who found a much smaller percentage of heavy smokers (28.4%) and a comparatively high percentage (41%) of nonsmokers and mild smokers among 757 cases of lung cancer for which data on smoking habits were obtained. Denk stated

† References 1128 and 1129.

that the American observations concerning a relation between a greater consumption of cigarettes and the likelihood of the development of bronchial carcinoma do not hold true to the same extent in Austria.

Smithers [1141] cautioned against advancing extravagant claims on the significance of cigarette smoking in the causation of lung cancer and pointed to the possible role of urban air pollution as a likely factor.

While some of the not inconsiderable differences in the relative percentages of smokers of various degrees are doubtlessly due to the use of different standards in the classification used, this explanation, however, does not hold for the proportion of nonsmokers listed by the different investigators. The percentage range for nonsmokers is from 1.3 to 14.6% for the various lung cancer groups and from 8.8 to 30.5% for the control groups. These discrepancies suggest the existence of differences in the basic composition of the human material evaluated. The validity of this concept also is supported by the fact that the various investigators noted rather widely varying proportions of adenocarcinomas in males and females in their respective series. The histologic type of pulmonary cancer is predominantly of the epidermoid variety among males, while a considerable proportion of these tumors among women are of the adenocarcinomatous type (36.4% in females, 4.5% in males [Gsell [1136]]; 52% in females, 18% in males [Proceedings of the First National Cancer Conference]; 13.7% in females, 6.7% in males [Mason [1142]]; 52% in females, 0.6% in males [Wynder and Graham [1113]]). It was noted also that a history of heavy smoking was less often elicited from patients with adenocarcinoma than from those with epidermoid carcinoma (Gsell [1136]; Wynder and Graham [1113]).

The apparent lack of uniformity in the human material analyzed by the various authors is further demonstrated by the appreciable differences in the sex distribution of lung cancers reported at different times, from different regions, and by different investigators. The male-to-female sex ratio fluctuates between 2:1 to 20:1 (Hueper [1143]). It is noteworthy, however, that the uniformly observed prevalence of lung cancer among males has, in general, become in recent years even more pronounced than in former decades. This observation strongly militates against a predominant causal role of cigarette smoking in the production of lung cancer, because all previous experience in the field of occupational cancer indicates that, given the same type of carcinogenic exposure for both sexes and, at the same time, an increasing equalization of the intensity of exposure, there occurs a narrowing of the gap in incidence rates of the two sexes, and not a widening, which is what actually exists. This interpretation of the diverging, sex-related frequency trends is not fundamentally affected by the statement that the interval between the start of tobacco smoking and the appearance of a lung cancer is between 20 and 40 years (Wynder and Graham [1113]; Ochsner, DeCamp, DeBakey, and Ray [1116]; Schrek, Baker, and Ballard [1112]). Even if women may not have indulged on a large scale in tobacco smoking some 30 years ago, there can be little doubt that the cigarette-smoking habit has made, during this period, much greater strides among women than among men.

The purely statistical approach leading to the assumption of the existence of causal relations between two coincidental events and trends is thus in urgent need of supporting biologic evidence. It is for this reason that the negative statistical correlation between pulmonary cancer frequency and cigarette smoking recently reported by Dungal from Iceland has added relatively little to the basic issues. Dungal pointed

out that there were among 1,939 autopsies performed from 1939 to 1948, with 417 cancers of all sites, only 12 pulmonary cancers (2.9% instead of 10% to 20% in the United States and 12% in Switzerland), that tobacco, especially in cigarettes, was not particularly popular in Iceland until 1939, and that tarring of the roads, which had extensively been done since 1920, had not exerted any influence on lung cancer frequency.

(*b*) Experimental Tobacco Cancer: Attempts to produce cancer with tobacco tar in experimental animals began many years before any relation between tobacco smoking and lung cancer was suspected—that is, at a time when the claims of a causal relationship between cancer and tobacco smoking were still limited to cancers of the lip, tongue, mouth, and larynx.‡

(1) Skin applications of tobacco tar. Wacker and Schmincke,[1146] as well as Helwig,[1147] using tobacco tar extracts which they applied to the ears of rabbits and to the skin of mice, respectively, produced only ulcers with epithelial proliferations, but not cancers. Similarly negative were experiments of Hoffmann, Schreus, and Zurhelle,[1148] who applied denicotinized tobacco tar to the skin of mice for 80 days, and experiments by Cooper and co-workers,[1149] who used the same technique for 23 months and observed a single skin cancer. Roffo § and Chikamatsu [1154] reported the production of cancroids in the ears of a few rabbits after prolonged painting with tobacco tar. These claims were confirmed by experiments of Lü-Fu-hua,‖ but cancers of rabbit ears were seen only when he simultaneously used intravenous cholesterol injections in one ear and painted the other ear with coal tar. These observations of Lü-Fu-hua on cholesterolized and coal-tar-treated rabbits were successfully repeated by Schürch and Winterstein,¶ who, in turn, failed to produce skin cancers in mice receiving skin application of tobacco tar and various tobacco tar fractions having different boiling points.

Sugiura subsequently succeeded in producing a solitary squamous cell carcinoma in a mouse painted with tobacco tar distilled at temperatures between 500 and 900 C. Tobacco tar distilled between 100 and 500 C. proved to be noncarcinogenic for mice when applied to the skin. Both distillates were administered in an oily mixture to the ears of rats and rabbits for 52 to 95 weeks, without tumor formation. Flory [1160] repeated the application of tobacco tar distillates of different boiling points (130 to 350 and 350 to 700 C.), as well as of pipe tobacco tar, to the ears of rabbits and obtained, in a considerable proportion of the animals, papillomas and carcinomatoid tumors but not carcinomas. Several squamous cell carcinomas were found in mice after the application of these tars.

(2) Inhalation of tobacco tar fumes. The first attempts to produce cancer of the lung in experimental animals by the inhalation of tobacco smoke were made by Mertens,# using mice which were exposed in glass jars to tobacco smoke injected into these vessels. From the first set, of 125 mice, 2 developed "lung cancer," but in both instances the cancers most likely were of "spontaneous" origin. When this experiment was repeated, no lung tumors were obtained. Likewise negative were

‡ References 1144 and 1145.
§ References 1150-1153.
‖ References 1155 and 1156.
¶ References 1157-1159.
# References 1161 and 1162.

similar experiments of Lorenz and co-workers,[1163] in which mice inhaled tobacco smoke introduced into a closed container. The strain A mice exposed to tobacco fumes showed the same incidence of spontaneous lung tumor as did control animals. In Campbell's [1164] experiments, in which a similar technique of inhaling tobacco smoke was used, there followed a minor increase of lung tumors in the test series over that of the controls.

A more direct and drastic method for introducing tobacco tar into the lungs of experimental animals was chosen by Roffo, who injected this material directly into the lungs of rats and obtaining, in one rat, four small squamous cell carcinomas.

A critical evaluation of the total experimental evidence permits the conclusion that tobacco tar obtained at various distillation ranges is of very weak, if not doubtful, carcinogenicity to the skin of mice and produces apparently only carcinomatoid tumors in the ears of rabbits when applied over long periods of time. The inhalation of tobacco smoke released into the atmosphere failed to produce lung cancers in mice. The unconfirmed positive claims of Roffo have been disregarded in reaching these conclusions.

(3) Demonstration of known carcinogens in tobacco smoke and tar.

Among the know carcinogenic chemicals which may occur in tobacco smoke or tar, carcinogenic aromatic hydrocarbons and arsenicals have to be considered. The carcinogenic aromatic hydrocarbons might be formed during the combustion of the tobacco, while arsenicals might occur only in those tobacco tars and fumes which are generated from tobacco containing arsenical insecticide residues.

Although Roffo * asserted he had demonstrated 3,4-benzpyrene in tobacco tar by spectroscopic methods, this claim has remained unconfirmed by several very reliable investigators.†

While the failure to demonstrate 3,4-benzpyrene in tobacco tar does not exclude the possible presence of other carcinogenic chemicals in this material, it, nevertheless, is an observation which is noteworthy, because 3,4-benzpyrene seems to be one of the common carcinogenic combustion products of carbonaceous matter of many kinds.

In view of these negative findings for carcinogenic hydrocarbons in tobacco tar, some investigators recently have favored the concept that the alleged carcinogenic effect of tobacco smoke upon the respiratory tract depends, at least in part, upon the inhalation of arsenic present in the tobacco as an insecticide residue and volatilized during the smoking process (Doll and Hill [1175]; Goulden, Kennaway, and Urquhart [1165]). In fact, rather appreciable amounts of arsenic can be demonstrated in tobacco and in tobacco smoke, especially in the American variety. Gross and Nelson [1166] found that the arsenic content of cigarette tobacco of five brands ranged from 9.7 to 36.3 ppm, that of cigars from 8.3 to 48.4 ppm, and that of pipe tobacco from 26.0 to 50.0 ppm. Thomas and Collier [1167] noted that the range of the arsenic content of cigarette tobacco was from 35.4 to 114 ppm, that of cigars from 13.2 to 29.5 ppm, and that of pipe tobacco from 22.7 to 42.8 ppm. The reason for the marked variations in different samples and various types of tobacco is undetermined. However, different climatic conditions and methods of cultivation

* References 1150-1153.

† References 1149, 1157-1159, and 1172-1174.

and processing of tobacco in various parts of the United States and the different use to which the various types of tobacco are subsequently put in the production of tobacco goods may have a decided influence in this respect.

In more recent studies of cigarette tobacco by Daff and Kennaway,[1133] three American brands gave an arsenous oxide content ranging from 25 to 47 γ; two English brands had one ranging from 50 to 55 γ; eight Turkish brands had one ranging from 0 to 4.1 γ; for a French brand the range was 0.5 to 1.5 γ, and for a Rhodesian brand, 1.8 to 4.1 γ. Popp[1168] found that Palatinate tobacco contained 5.1 ppm of arsenic; Macedonian cigarette tobacco 0.7 ppm; Java tobacco 0.33 ppm, and Brazilian tobacco 4.6 ppm. Oliver[1169] reported that English pipe tobacco contained 32 ppm of arsenic (as metal), cigarettes 68 ppm, Jamaica cigars 30 ppm, and Havana cigars 170 ppm.

The observations show that the concentration of arsenic in cigarettes, cigars, and pipe tobacco is very variable, depending upon the brand as well as upon the country of origin. The tobacco of American derivation, and smoked in the United States, Canada, Norway, and England, has by far the highest arsenic content (24 to 106 γ of As_2O_3 per gram of tobacco), while tobaccos grown in the eastern European countries and Turkey have, as a rule, a low arsenic content (0.0 to 4.3 γ of As_2O_3 per gram of tobacco). This type of tobacco is used in cigarettes made in Austria, France, Poland, and Bulgaria.

In judging the degree of arsenic hazard which may result from the smoking of arsenic-containing tobacco, Daff and Kennaway, Thomas and Collier, and Gross and Nelson ascertained that between 7.0 and 26% of the arsenic present in the tobacco is volatilized and may be inhaled during the smoking process. Remington,[1176] however, placed this portion as high as 50%. Daff and Kennaway expressed the degree of potential hazard by the following calculation:

> If a person smokes 50 cigarettes with a mean arsenic content of 50 μg and 15 per cent of this escapes, he has volatilized 0.375 mg. As_2O_3, which is the amount contained in 0.0375 cc. of strong iodine (Fowler's) solution U. S. P. (official dose 0.125 to 0.5 cc.).

In a recent, very illuminating study of the relation of cancer of the lung to tobacco as grown and smoked in different countries, Daff, Doll, and Kennaway[1134] have unearthed important observations which strikingly demonstrate the complexity of the problem and show "that the arsenic content of tobacco has not provided any simple and exclusive explanation of the association between cigarette smoking and this form of cancer." Analyzing first the data provided by Saglam, Schwartz, and Yenerman from Istanbul, Turkey, where for over five decades tobacco has been consumed almost wholly in the form of cigarettes and where there have been many heavy smokers among women, they found that there had been a considerable rise in lung cancer frequency during the past 50 years, according to clinical and anatomic-pathologic statistics (increase in clinical material, 12 times; in pathologic material, 4.1 times). However, the male-female ratio changed from 6:1, during 1935 to 1939, to 8:1, during 1949-1950, although cigarette smoking has been a habit indulged in by Turkish women for many decades. The tobacco consumption, expressed in pounds per head, increased from 1.21, in 1925, to 1.9, in 1949. Since the tobacco consumption stood in 1935 at only 1.55 lb. per head, and in view of the long lag period in the development of lung cancer, it is most unlikely that the increase in lung cancer frequency in Istanbul has any relation either to tobacco consumption or to its arsenic content. This view is supported by the sex ratio, which is, as in

many other countries, markedly and increasingly in favor of males, despite the long-established smoking habit among Turkish women. Saglam also, therefore, rejected cigarette smoking as a factor in the incidence of bronchial carcinoma.

Another illustration of the apparent lack of significance of arsenic in tobacco in relation to the causation of lung cancer and its recent increase seems to be provided by the autopsy data from Ljubljana, Yugoslavia, provided by Kosir. During the period of 1925 to 1939 lung cancer represented 7% of cancer of all sites in males coming to autopsy and 1.5% of cancer of all sites in females of the same type, while during 1940 to 1949 these figures stood at 15% and 2%, respectively. Tobacco is smoked principally in cigarettes of oriental type, with a low arsenic content. The increase in lung cancer frequency, thus, was disproportionately large for males.

The data provided by Daff, Doll, and Kennaway on Switzerland by von Meyenburg have been complemented by those recently given by Gsell.

According to Gsell,[1136] there has taken place in recent years a marked increase in the consumption of cigarettes (ten fold between 1924 and 1949), but the main tobacco product consumed in Switzerland had remained the Stumpen, a medium-sized cigar without a tip. There were 48 Stumpen and cigar smokers, and only 30 cigarette smokers, among Gsell's 87 cases. The absolute number of lung cancer cases rose in males from 68, in 1905 to 1909, to 2,058, in 1945 to 1949 (20.7 times), while the corresponding increase in female cancer deaths was from 59 cases, in 1905 to 1909, to 421 cases, in 1945 to 1949 (7.1 times). During the same period there occurred a shift of the male-female sex ratio from 2:1, in 1905 to 1909, to 4.9:1, in 1945 to 1949. The lung cancer death rate per million in 1931 stood at 66 for males and at 14 for females, while in 1947 it was 183 for males and 34 for females. The arsenic content of tobacco of Swiss cigarettes was of intermediate order (3.1 to 12.0 γ of As_2O_3 per gram). Since during the critical period of lung cancer increase for which Swiss tobacco consumption figures are available (1924 to 1935) the main use of tobacco was in the form of cigars, Stumpen and Toscani, one must assume that the majority of the lung cancers observed during the years 1940 to 1949 cannot be attributed to the smoking of cigarettes, if smoking at all had any causal connection with their development. That the existence of such a relation is not likely is again attested by the divergent trends in the sex distribution of lung cancer, which, according to past experience on this point from the field of occupational cancer, would display a tendency toward equalization whenever members of both sexes are exposed to the same environmental carcinogenic agent. That this principle also applies to tobacco smoking is evident from the recent statistical studies of Doll and Hill, who ascertained that the risk of developing cancer of the lung is the same in men and women, apart from the influence of smoking.

The importance of this argument is strikingly illustrated by the information supplied to Daff, Doll, and Kennaway by Kreyberg concerning the lung cancer incidence in Norway during the past 20 years. Apart from the fact that deaths from lung cancer were higher in urban than in rural districts of Norway, there occurred during the two decades a definite rise in pulmonary cancer frequency from about 20, in 1930, to approximately 157, in 1948. The rise was more marked in urban areas than in rural ones and affected males to a higher degree than females. While thus, the Norwegian experience followed in this respect the usual pattern, it differed fundamentally from it in its sex distribution. During the first six years of

the survey period, lung cancers were apparently as frequent in females as in males, and thereafter the rise of the rates in females fell somewhat below that of males. This observation permits only one interpretation when analyzed in the light of the existing facts, that the epidemiologic behavior of lung cancer as to sex distribution was probably influenced in Norway by environmental factors which were not active at all, or were not active to the same degree, in the other countries investigated. Since the population of Norway has smoked predominantly American-made tobacco, this deviation from the common epidemiologic pattern does not support the view that tobacco smoking in Norway, or in any of the other countries, was a deciding and important factor in determining lung cancer epidemiology during the past 50 years.

In the various arguments advanced purportedly favoring the cigarette-smoking role in the causation and rise of lung cancers, much is made of the existing parallelism between the increase of lung cancer frequency and the increased consumption of smoking tobacco, particularly cigarettes, during the last 25 to 50 years. In the graphic presentation of these two developments the relative events of annual rise of lung cancer rates and of tobacco consumption are invariably synchronized, whereas in fact these events have a distinct heterochronicity because of the long latent period of lung cancer, which has been estimated for smokers to range between 20 and 40 years. The lung cancer cases observed in 1950, for instance, therefore have no causal connection with the tobacco consumption of the same year but more likely are related, if at all, with that recorded for 1920 to 1935. Is is evident from this consideration that the so-called "parallelism" as presented by synchronized graph lines gives a definitely distorted expression of any possible hypothetical relation between lung cancer and tobaccoism (Figs. 5, 6, and 7).

Distinctly disconcerting in this respect is also the obvious disagreement of different investigators as to the relative role which cigarette smoking, on the one hand, and the smoking of pipe tobacco and cigars, on the other hand, allegedly play in the causation and rise of lung cancer. While Levin and co-workers contended that only cigarette smoking, but not pipe and cigar smoking, reveals a positive statistical correlation, Mills and co-workers emphasized that all three forms of smoking are equally guilty, while Wynder and Graham, Gsell, and, to some degree also, Doll and Hill assess the individual smoking habit by including all types of smoking. While Doll and Hill contemplated the possibility that pipe smoking may be less lung-cancer-inducive than cigarette smoking because, in their opinion, pipe smokers smoke less tobacco than cigarette smokers, it may be well to consider the fact that many cigarette smokers discard their cigarettes after a few puffs and that, therefore, the assessment of the degree of cigarette smoking may more easily become exaggerated, while that of the pipe and cigar smoker may become underestimated. Gage, indeed, stated that one study strongly indicated that the average cigarette smoker consumes less tobacco per day or year than a cigar smoker or tobacco chewer.

Of undoubted importance seems to be another statement of Doll and Hill in which they note that inhaling of cigarette smoke did not convey any increased lung cancer liability. This is an observation which cannot be reconciled with facts established for determining occupational cancer incidence. Whenever the intensity and duration of exposure to an occupational carcinogen increases, there rises the cancer incidence rate among the exposed population group. There is no plausible reason to assume that the inhalation of allegedly carcinogenic tobacco smoke would

be exempted from this rule. The complete lack of even minor increase of laryngeal cancer during the past five decades, although the larynx forms a part of the smoke tract, also militates against the tobacco-smoking theory of lung cancer.

It may be concluded that the existing evidence neither proves nor strongly indicates that tobacco smoking, and especially cigarette smoking, represents a major, or even predominating causal factor in the production of cancers of the respiratory tract or is the main reason for the phenomenal increase of pulmonary tumors during recent decades. If excessive smoking actually plays a role in the production of lung cancer, it seems to be a minor one if judged from the evidence on hand. However, it may be well to remember, in this connection, the concluding statement of Doll

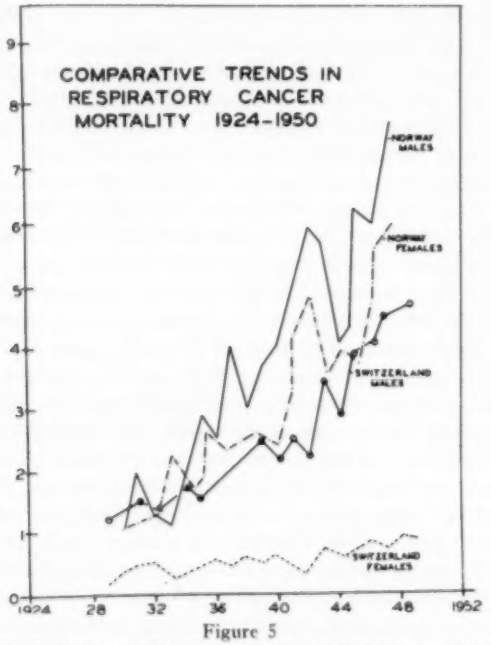

Figure 5

From Daff, M. E.; Doll, R., and Kennaway, E. L.: Brit. J. Cancer **5**:1, 1951; published by H. K. Lewis & Co., Ltd., London.

and Kennaway that "the study of the relation between the national consumption of tobacco and the national incidence of cancer of the lung has scarcely begun."

Special mention, however, may be made in this connection of the frequent occurrence of cancer of the palate in the Northern Cicars in India. These people have the peculiar habit of smoking cigars, for economical reasons, with the lighted end inside the mouth. The epithelial lining of the palate undergoes leucoplakic changes at the site where the lighted end lies in juxtaposition. Irregularly oval or crescent ulcers develop, which may undergo malignant change of the nodular or ulcerative type (squamous cell carcinomas). Children of 6 to 7 years begin to acquire this habit, and the average age when the cancer shows is 42.4 years.‡ It has

‡ References 1170 and 1171.

been reported that a similar habit of inverted smoking of cigars is practiced by Panamanian Indians, where it is responsible for the occurrence of leucoplakia. It is obvious that, under the circumstances of exposure, the tissues of the palate not only are exposed to the tobacco smoke but sustain also repeated burns, with carbonization of the tissue.

J. Specific Aspects of Environmental Cancers

1. *Population Types and Sizes of Groups Exposed to Industrial Carcinogens.*— It is often asserted that the scope of the occupational cancer problem is relatively limited because of the rather small number of cases of cancer recorded as being due to occupational agents, and the low rate of occupational disability attributable

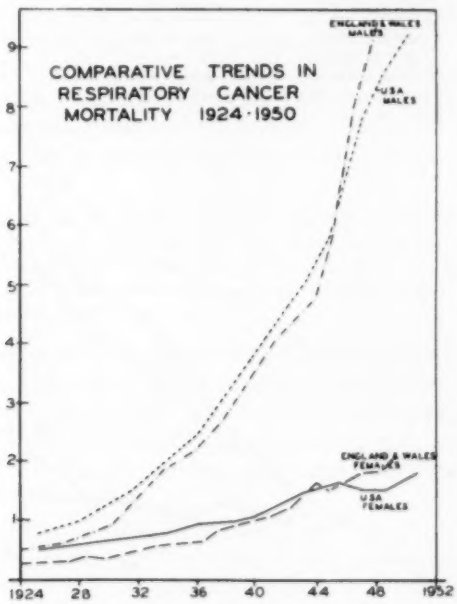

Figure 6

to cancers of this origin. In assessing the real value of such assertions, it may be pointed out that at the present time there do not exist in the United States any worth while data on the incidence or frequency of occupational cancers in our industries, with the possible exception of the information recently obtained on the occurrence of lung cancer among chromate manufacturers. One may contemplate the fact in this connection that from 100 to 250 cases of industrial skin tumors are annually notified with the Chief Inspector of Factories of England and Wales, while scarcely any have been reported during the last two decades from this country, although our industries producing, handling, and consuming tar, pitch, and mineral oils employ a considerably larger number of workers than those in Great Britain. Despite the fact that the first discovery of cancers of the skin among workers of paraffin-pressing operations in oil refineries was made in 1910 in this country, and

that additional cancer cases of this origin were placed on record during subsequent decades from refineries in Ohio, Illinois, and Indiana, no new cases have been reported during the last 20 years. It is a well-established fact, on the other hand, that tar, pitch, and oil cancers have been observed among American workers during this period. Similarly, it is now 15 years ago that the last communication as to the

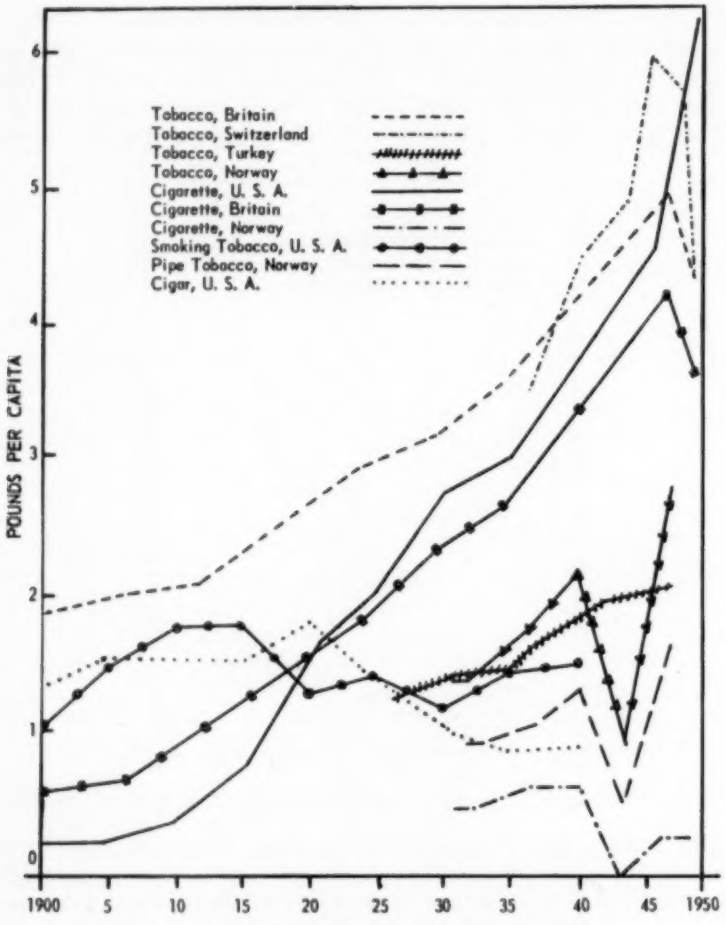

Fig. 7.—Tobacco consumption per capita, in pounds.

number of aromatic amine cancers of the bladder among American dye workers appeared in print, although the number of such cases found among this worker group has more than doubled since 1938.

It seems that a much more adequate measure of the significance of occupational, and thus, in part, also of environmental, carcinogens in the production of human cancer is represented by the number of persons who become for occupational or other reasons exposed to specific carcinogenic agents of industrial origin. The pamphlet

"Environment and Health," published in 1951 by the Public Health Service, recorded the following information on numbers of American workers exposed to chemicals entailing health hazards, including occupational cancers; asbestos dust, 34,949; arsenic and compounds, 32,855; benzene, 28,121; coal tar, pitch, creosote, petroleum derivatives of various kinds and their numerous products of incomplete combustion and distillation; blacksmiths, forgemen, and hammermen, 87,166; locomotive engineers, 72,396; locomotive firemen, 48,851; machinists, 521,093; mechanics and repairmen, 974,352; stationary engineers, 320,285; machinists' apprentices, 14,198; firemen, 165,031; painters, 100,726; oilers, 39,498; furnacemen, 33,932; roofers, 32,720 (total of approximately 2,500,000 workers); metal dust, welders, 139,281; metal buffers, 45,035; grinders, 45,902; filers, 10,952 (total about 240,000).

It is apparent from this partial listing of occupational groups with potential industrial cancer hazards that the total number of persons in the United States having contact with such agents is considerable. There can, therefore, be little doubt that the actual number of persons developing cancers of various organs because of such exposures is definitely much larger than indicated by published records.

2. Age Distribution of Environmental and Occupational Cancers.—Willis [1177] noted:

> We now know that cancer is mainly a disease of the elderly, not because senile tissues are "predisposed" to cancer, as was once supposed, but because of the usually long latent periods elapsing between the application of carcinogenic stimuli and the development of tumors. Occupational and experimental tumors show that these periods often occupy large fractions of the life spans of the affected animals.

The validity of this statement is supported by the data in Table 23.

The observations from the field of environmental and occupational carcinogenesis not only demonstrate that the basic cancerization process antedates often by many years the time at which the cancer becomes manifest, but also show that the age at onset of exposure and the intensity of contact with the carcinogenic agent determine the manifestation age.

When children from 4 to 10 years old entered the profession of sweeps in England during the early part of the last century, chimney sweeps developed scrotal cancer at an average age of 30 to 40 years.[5] After this practice was discouraged by law, and sweeps did not start in the trade before the age of 16, the average age at which scrotal cancer in sweeps was observed rose to from 45 to 50 years at the end of the 19th century. With the subsequent introduction of improved hygienic conditions and technical procedures reducing the intensity of exposure to soot, the average age of sweeps with scrotal cancer increased to 61.9 years by 1935. The evidence clearly shows that the progressive and considerable increase in the average age of sweeps with scrotal cancer was directly dependent upon the later onset of exposure and on a reduction in the intensity of exposure. While sweeps still have a higher liability to scrotal cancer than the general population, the average manifestation age of scrotal cancer in sweeps is now identical with that of scrotal cancers of unknown etiology (Table 24).

The studies of Goldblatt,[1178] recently confirmed by Case, McDonald, and Pearson,[1179] on the relation of age of entrance of dye workers into the industry and the manifestation age of bladder cancer provide additional evidence in support of

TABLE 23.—*Latent Periods of Occupational Cancers*

Organ and Agent	Average Latent Period, Yr.	Range of Latent Period, Yr.
Skin		
Arsenic		
Medicinal	18	3-40
Occupational	25	4-46
Tar	20-24	1-50
Creosote oil	25	15-40
Mineral oil	50-54	4-75
Crude paraffin oil	15-18	3-35
Solar radiation	20-30	15-40
X-radiation	7	1-12
Lung		
Asbestos	18	15-21
Chromates	15	5-47
Nickel	22	6-30
Tar fumes	16	9-23
Ionizing radiation	25-35	7-50
Bladder		
Aromatic amines	11-15	2-40
Nasal Cavity and Nasal Sinuses		
Nickel	11	3-26
Isopropyl oil	10	6-16
Radioactive gases and dust	25	19-32
Bones		
Radium, mesothorium	..	10-25
Hematopoietic Tissues		
Benzene	..	3-19
Ionizing radiation	..	3-15

TABLE 24.—*Age Distribution of Scrotal Cancer in Chimney Sweeps*

Age, Yr.	No. Cases	
	1892	1935
25-35	4	1
36-45	7	6
46-55	14	18
56-65	4	33
66-75	..	28
76-85	..	17
Average age	45-50	61.9

TABLE 25.—*Age Distribution of Aromatic Amine Cancer of the Bladder According to Age at Entrance of Employment* *

Age at Entrance	Age at Death from Bladder Cancer					
	30-39	40-49	50-59	60-69	70-79	Total
14-29	5	13	8	0	2	28
30-40	1	2	10	5	0	18
41-52	0	0	1	8	4	13

* From Goldblatt (Goldblatt, M. W.: Brit. M. Bull. **4**: 405, 1947, published by the British Council, London).

the dogma [1181] that not physiologic aging processes but age at onset of exposure to carcinogens is one of the factors which determines the manifestation age of human cancers (Table 25).

A similar effect of the age at onset and of the intensity and the duration of exposure to an environmental carcinogenic agent upon the average manifestation age of the resulting cancer is evident in arsenical cancers of the skin. While the average age of skin cancer patients is about 60 years, approximately one-third of 115 medicinal arsenical cancers were seen in patients less than 40 years old, and more than 60% were not older than 50 years. Likewise, the average age of persons with occupational x-ray cancer of the skin is in the group of 41 to 66 years. Occupational cancer of the bladder of chemical or parasitic origin also is characterized by an age incidence which favors the younger age groups, below 50 years of age

TABLE 26.—*Age Distribution of Occupational Cancers*

Organ and Agent	10-30		31-40		41-50		51-60		61-70		71 and Over		Total No.
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Skin													
Arsenic	10	8.7	28	24.3	35	30.4	24	20.9	17	14.8	1	0.9	115
Pitch	6	5.1	14	12.2	34	29.6	38	33.1	20	17.3	3	2.7	115
Tar	..	...	6	7.5	14	17.5	15	18.7	32	40.0	13	16.2	80
Paraffin	..	...	6	5.9	30	28.8	38	34.8	29	27.6	3	2.9	106
Shale oil	..	...	12	17.8	22	32.3	23	33.8	6	8.9	5	7.3	68
Solar radiation	2	0.9	8	3.6	24	10.7	58	25.8	76	33.8	56	25.0	224
X-radiation	..	...	18	51.4	9	26.1	6	17.1	0	0.0	1	2.9	35
Control	13	1.8	74	10.4	125	17.6	165	23.2	174	24.5	157	22.2	709
Lung													
Asbestos	..	...	2	11.8	7	41.2	6	35.3	1	6.0	1	6.0	17
Chromate	1	1.0	11	11.0	22	22.0	34	34.0	21	21.0	1	1.0	100
Tar	..	...	7	33.3	12	57.1	1	4.8	1	4.8	..	...	21
Ionizing radiation	6	5.3	30	26.7	38	33.6	29	25.7	10	8.7	..	...	113
Control	99	5.8	222	12.2	444	24.5	609	33.6	356	19.6	62	3.4	1,792
Bladder													
Aromatic amine	8	3.8	40	19.0	63	30.0	81	38.6	17	8.1	1	0.5	210
Control	2	0.8	21	8.1	59	22.7	85	32.8	63	24.2	29	9.4	259

(aromatic amine cancer, 52.8% of all cases in persons less than 50 years old; schistosomiasis cancer, mainly in patients 30 to 40 years old), as contrasted with an age distribution of more than 50 years for 65% of all cryptogenetic bladder cancers.

The evidence presented in Table 26 shows a shift into younger age groups for skin cancers caused by arsenicals, pitch, paraffin oil, shale oil, and x-radiation, for cancer of the lung induced by tar and ionizing radiation, and for cancers of the bladder produced by betanaphthylamine and benzidine.

Additional evidence militating against the fundamental importance of age factors in the production of many human cancers is provided by observations on the epidemiology of penile cancer. The large-scale Jewish experience with circumcision on the eighth day of life indicates that this procedure affords complete protection

against a subsequent development of penile cancer, and the studies of Schrek and Lenowitz [1180] on circumsized American white persons and Negroes suggest that circumcision performed during the first six years has a similar protective effect. Circumcision at a later age (6 to 35 years), on the other hand, does not result in any significant difference in the incidence of penile cancer between circumcised and noncircumcised persons. Since the interval between circumcision and the appearance of penile cancer ranges from 8 to 40 years, with an average interval of 23 years, it is evident that the specific carcinogenic exposure in penile cancer apparently takes place during the first 10 to 15 years of life.

The high death rate of cancer of the lung among miners of radioactive ores, which stands at some 70% of all deaths for the Schneeberg miners and at some 40% for the Joachimsthal miners, cannot be attributed to the existence of a "preferential" type of aging among these population groups. The progressive rise in the incidence of pitch warts and cancers among English pitch workers, from 17% of the workers after 1 to 5 years of exposure to 100% after more than 40 years of exposure, provides an additional illustration of the lack of importance of senescent changes in the causation of occupational cancers.

The occurrence of cancers in infants and children and their difference in distribution by site and in histologic character from cancers seen in adults have given rise to the concept that cancers in the young are not caused by exogenous factors but are due to congenital developmental mechanical (embryonic rests) or endogenous hormonal disturbances sustained during fetal life.§ The reported increase of cancers in childhood during the last 25 years [1184] suggests, however, that other factors may actually be active in their causation.

A number of clinical and experimental observations made during recent years showed that exogenous chemical and infectious agents may penetrate the placental barrier and that some of them have elicited fetal defects, malformations, and poisonings.‖ These agents include several which have produced tumors in adult experimental animals (urethan, thiouracil, trypan blue). Larsen [1189] and Klein [1191] recently demonstrated, moreover, that the administration of urethan to pregnant mice resulted in an early and increased appearance of pulmonary adenomas, indicating a transplacental transmission of a carcinogenic agent from the mother to the fetus.

The potential significance of these observations for the human cancer problem [1192] was recently ably discussed and analyzed by Peller [1193] on the basis of epidemiological data on cancers in childhood and the physiologic conditions peculiar to fetal life. Peller concluded from his consideration of the evidence that a transplacental exposure of the fetus to exogenous carcinogenic agents may readily account for the incidence, topographical distribution (brain, kidney, hematopoietic tissues), and anatomical types of cancers observed during this age period.

In addition to a possible transplacental mechanism of transmission of carcinogens to the fetal organism, a transfer of such agents through human maternal milk may also play a role in the production of cancers in infants and children. It is known that a considerable number of drugs (arsenic, barbiturates, nicotine, bromides, sulfonamides, iodine, phenolphthalein) are excreted with the human milk,¶ while

§ References 1182 and 1183.

‖ References 393, 1185-1189, and 1192.

¶ References 1194-1199.

selenium has been found in the milk of rats.[1200] Some of these agents are known or suspected carcinogens. Nurnberger and Lipscomb [1199] cautioned against the oral administration of radioiodine in tracer doses to lactating women because the concentration of the radioactive iodine in the maternal milk is sufficiently high to allow a sizable and hazardous uptake in the thyroid of the baby. While the relative rarity of reports concerning the poisoning of infants by occurrence of exogenous toxic chemicals excreted with the maternal milk seems to militate against the practical importance of such potential connections, it is well to remember two established facts: (1) There does not exist a direct relation between the toxicity of a chemical and its carcinogenic properties, and (2) carcinogenic effects can be elicited with doses which are distinctly below acute or chronic toxic doses. Some consideration may also be given to observations attesting that effective carcinogenic exposures do not need to be continuous, are often intermittent or, occasionally, singular, and that subsequent contacts with specific cocarcinogenic or nonspecific irritative factors may exert a provocative effect upon the carcinogenically sensitized tissue and thus act as realization factors, possibly causing the appearance of cancers years after a specific carcinogenic exposure.

3. *Classification of Carcinogens.*—An analysis of the action mechanism of the various recognized, suspected, or potential human carcinogens supports the view that the majority of cancers develop at sites where, for some reason, the most intense or most prolonged exposure to the carcinogen takes place. The following types of mechanisms determining the distribution of environmental cancers may be distinguished.

(*a*) Cancers Developing at Sites of Primary Contact: To this group belong the cancers of the skin resulting from cutaneous exposures to substances such as coal tar, petroleum oils, creosote oil, soot, and similar combustion and high-temperature distillation products of carbonaceous matter, as well as to ultraviolet and ionizing radiation; to this group also belong the cancers of the nasal cavity, nasal sinuses, larynx, and lung elicited by the inhalation of arsenicals, chromium and nickel compounds, beryllium (?), asbestos, isopropyl oil, tarry matter, and radioactive gases and dust. The cancers of the connective, bony, and hematopoietic tissues following exposures to penetrating ionizing radiation may be included in this group of primary contact cancers.

(*b*) Cancers Developing at Sites of Selective Deposition: Arsenical cancers of the skin; osteogenic sarcomas following ingestion of radium and/or mesothorium, or after inhalation of beryllium (?); leukemia following contact with benzene, and thyroid carcinoma following radioactive iodine administration, and leukemia subsequent to radioactive phosphorus medication may be included in this group.

(*c*) Cancers Developing in Organs with Special Functional or Toxic Affinity for Carcinogens: Representatives of this group are almost exclusively caused by carcinogens of potential importance only so far as humans are concerned, and thus have been observed mainly in experimental animals. Cancers of this type are the tumors of the liver developing after exposure to various azo dyes, aminofluorene compounds, chlorinated hydrocarbons, dulcin, certain alkaloids, thiourea, and selenium; the cancers of the breast and uterus subsequent to excessive exposures to estrogens, and cancers of the thyroid following the prolonged administration of thiouracil compounds.

(*d*) Cancers Developing in Organs of Excretion of Carcinogens: Cancers of the bladder, ureter, and kidney observed in patients and experimental animals having cutaneous, ingestive, or respiratory contact with certain aromatic amines and azo compounds due to the presence of carcinogenic material in the urine belong to this group.

(*e*) Cancers Developing on the Basis of Functional Abnormalities Due to Certain Dietary Deficiencies and Representing a Type of Indirect or Secondary Environmental Carcinogenesis: Cancers of the hypopharynx and of the liver noted among population groups subject to a diet deficient in vitamin B complex and protein, as well as cancer of the liver in rats kept on a choline-deficient or ethionine-containing diet are members of this group. There exists a good deal of circumstantial experimental evidence suggesting that an indirect carcinogenic mechanism may also be active in the development of liver tumors by the various hepatotoxic chemicals, of thyroid tumors by antithyroid agents, and of leukemias, myelomas, and lymphosarcomas by myelotoxic chemical factors, especially those possessing an allergenic, agranulocytic action.

4. *Heredity and Constitution.*—Despite the large amount of biostatistical work on cancer heredity done with cancers of man and experimental animals, there is astonishingly little positive and valid evidence indicating that hereditary factors play a primary and/or important role in the production of the majority of human cancers. An indiscriminate application of observations made on selectively inbred strains of mice has been instrumental in giving to hereditary factors an exaggerated and distorted significance as immediate causes of human cancer. The apparent fallacy of this procedure has become strikingly obvious with the demonstration of a virus-like "milk factor" in the milk of mice transmitting the "hereditary" tendency to cancers of the breast to the offspring ingesting this exogenous agent.

While it may be conceded that there exist a few rare cancers which display hereditary tendencies, at least one of them (cancer of the skin in xeroderma pigmentosum) depends in its direct causation on the primary action of exogenous ultraviolet radiation. Although xeroderma pigmentosum is due to an inherited hypersensitivity to solar radiation prevalent among some inbred family groups, this fact would scarcely justify the conclusion that the ordinary type of solar cancer of the skin is primarily an inherited manifestation of the host organism which is activated by a specific or nonspecific exposure to solar rays, acting in the role of a trigger mechanism. Such an inversion or perversion of normal reasoning would have to disregard the fundamental fact that without solar radiation there is no cancer, irrespective of the constitution of the individual.

This conclusion is not negated by the well-established fact that racial differences in pigmentation determine the relative susceptibility to cancer of the skin through an exposure to solar radiation or carcinogenic tars and oils of members of racial groups having varying degrees of melanin deposits, since these products do not seem to participate in the actual process of carcinogenesis, but act merely as nonspecific, although natural, screening agents. While doubtlessly an interbreeding of fair-skinned persons with dark-skinned ones would result in a human strain with increased resistance to the carcinogenic effects of solar radiation, such an application of genetic considerations to human cancer control has no practical merits or general appeal.

As to a significant hereditary factor in cancer of the female breast, there is a bewildering array of controversial statistical evidence. The allegation that the high liability of the radioactive ore miners in Schneeberg and Joachimsthal to cancer of the lung is, at least in part, a result of inbreeding of the local population of these mountainous regions represents pure speculation, which may conveniently fit a preconceived hypothesis but which has no support for available facts. The frequently cited occurrence of cancers of certain organs among members of the same family is not necessarily proof of a hereditary influence, because members of a family group are not infrequently exposed to the same environmental or occupational carcinogens (familial cancer of the scrotum among English chimney sweeps, familial cancer of the bladder in dye workers).

There exists, on the other hand, adequate evidence indicating that not only individual differences in the intensity and duration of exposure to a carcinogen, but also variations of individual inherited and acquired constitution play a role in determining the degree of susceptibility to environment cancer, as they do for any other environmental disease. Observations made with several environmental cancers (radioactive cancer of the lung, aromatic amine cancer of the bladder, pitch cancer of the skin) show that an almost complete obliteration of individual inherited or acquired constitutional susceptibility to exogenous carcinogens occurs in the presence of an overwhelming, high-intensity exposure. Whenever groups of persons become exposed to carcinogens of low potency or sustain exposures of low intensity and duration, there appears evidence indicating variations in the speed and character of the individual responses to the environmental carcinogen. Environmental cancers then display a scatter pattern of attack among members of exposed groups which parallels that seen with many infectious diseases under similar conditions of pathogenic contact.

It is unfortunate from the standpoint of cancer prevention that the presently available data on genetic factors related to cancer susceptibility or resistance are so vague and defective that with few exceptions, such as pigmentation, they are not amenable to any practical application. The frustrating situation present in this respect in regard to cancer, therefore, is similar to that existing for a number of infectious diseases, such as scarlet fever and poliomyelitis, which also exhibit in their epidemiologic pattern the influence of genetic factors. For these reasons, the emphasis of practical cancer prevention, therefore, rests for the time being on an effective elimination or reduction of contact with the known or suspected environmental carcinogenic agents.

5. *Control of Cancer.*—The development and institution of effective preventive measures for the control of human cancer have been impeded in the past by grossly defective information on, and appreciation of, the causes of cancer. This condition is in part due to the widely accepted dualistic concept which considers all cancers as a disease entity, having no or little connection with the diverse etiologic factors and the etiology-specific precancerous developmental symptoms and reactions. For this reason, scientific attention for many years has been focused mainly upon the study and diagnostic and therapeutic management of the biologic phenomenon "cancer," and relatively little effort has been made to explore the factors and events which lead to the creation of this so-called "spontaneous" anatomical manifestation.

The evidence presented demonstrates definitely that cancers are as little products of spontaneous generation as is a newborn baby. Both reaction products have very

specific creators, which determine their anatomical and dynamic properties, as well as their individual variations. The ultimate appearance of both products is preceded by a preparatory period, during which endogenous and exogenous influences may alter, modify, or terminate the progress of these particular cellular growths. It appears from this analogy that cancers represent anatomical end-products of a series of successive biologic events which are elicited by numerous and varied physical and chemical factors, and which seem to reflect changes in the reaction status of the affected organism. They may become localized in different sites and tissues, depending upon the causative factors and the route of contact, and display, despite many morphologic and biologic similarities, a wide range of structural and biodynamic differences.

A cancer may begin like an infectious disease, with the first exposure of the host to and his interreaction with a pathogenic agent. It appears from the great variety of specific agents which can elicit such reactions that these reactions must be as etiology-specific, and not host-specific, as are many of the cellular proliferative manifestations caused by infectious disease pathogens, since both represent fundamentally responses of the organisms to exogenous chemical factors. Since the etiologic specificity of cancerous reactions has been demonstrated for tumors induced by viruses, it may be assumed that the present lack of similar evidence for cancers caused by inanimate agents may be due to methodologic inadequacies rather than to fundamental biologic differences.

While the biostatistical approach to environmental and occupational cancer is an important one and has yielded in recent years valuable information to an increasing degree, the significant fact should not be overlooked that the great majority of these cancers was discovered and recognized without the help of such complex and complicated procedures. In fact, for most of the known occupational cancers reliable incidence figures are lacking, although some 175 years have passed since the first occupational cancer was described by Pott. As a rule, clinicians with keen analytic minds and sound medical judgment have been responsible for the basic observations on these undesirable products of our modern artificial industrial environment.

A wider and more inspired use of the medical approach to the environmental cancer problem is not only practicable and valuable, but also urgently needed. The clinical and pathologic data available are adequate to distinguish, in many instances, occupational cancers caused by certain agents from those of unknown origin, without having to rely on any supporting statistical evidence. Such occupational cancers display during their development (preparatory phase) and/or their cancerous phase symptoms and lesions which are etiology-specific and which thereby identify these cancers as belonging to a causal entity. Since not all persons exposed to a particular carcinogenic agent develop cancer, but may exhibit manifestations of nonmalignant nature characteristic of the carcinogenic pathogen, it is valuable at times to ascertain data on the existence or nonexistence of such associated symptoms among members of the population group from which the cancer patient originated. Through a skillful and competent use of such data, occupational cancers not only can be identified as to their etiologic background, but may be differentiated from cancers of the same site having a different causation.

Arsenic cancers of the skin, for instance, are frequently preceded by or associated with symptoms of chronic arsenicalism. Arguello noted such a combination in

90 of his 148 cases of endemic dietary arsenic cancer of the skin. Likewise, the majority of arsenic cancers of the lung displayed coexisting arsenic dermatoses and cutaneous cancers. The excessive incidence of cancers of the lung among English manufacturers of arsenical insecticides was accompanied by an abnormally high skin cancer death rate. The consistent absence of such etiology-specific cutaneous lesions among English nickel refinery workers and among German and Bohemian radioactive ore miners having an excessive liability to cancers of the lung and nasal cavity militates against the often-repeated contention that these respiratory cancers are attributable to occupational exposures to arsenicals.

Similar pathognomonic relations are demonstrable for the occurrence of cancers of the skin and of the lung among workers exposed to tar fumes (gas retort and coke oven workers) or mineral oil mists (paraffin pressers) and their frequent affliction by tar and oil dermatosis, warts, and papillomas. The anatomic and histologic manifestations of radiodermatitis and solar dermatitis provide adequate evidence of the causal agent active in the production of any cancer which might appear in the skin thus altered. The observation of a primary leucopenic phase followed by a hyperleucocytotic and leukemoid phase, which, in turn, precedes the leukemic state developing in response to exposures to benzene or ionizing radiation, as well as the demonstration of anemic, leucopenic, and leukemoid reactions among persons similarly exposed are of definite etiologic significance, apart from the fact that they are representative of the ambivalent properties of many carcinogens.

The need for the establishment of such etiology-specific symptomatic patterns for cancers of environmental origin is not only urgent for medical and medicolegal reasons, but also because of the growing realization that exogenous carcinogens possess species specific properties which limit the significance and usefulness of observations made on experimental animals.

There is an additional important reason for developing and establishing etiology-specific environmental cancer entities. The present-day method customarily used by epidemiologists and biostatisticians for determining causal relations between the exposure to environmental factors and the occurrence and incidence of cancers is based on the fallacious assumption that there exists an average, "normal," "intrinsic" liability to cancer for all members of a population group. Hence the existence of special cancer-producing factors for a particular population group is conceded only if it can be shown statistically that the incidence rate of cancer of a particular site is higher in the test group than that found for the population in general or for some other population group of similar age, sex, and race distribution, working or living under conditions different from those pertaining to the test group.

Since it has been demonstrated that the term "cancer" encompasses, evidently, a large number of different disease entities produced by diverse chemical and physical agents, and thus is actually a diffuse collective term, analogous to the term of "infectious diseases," this epidemiologic approach to the study of environmental causes of human cancer has as much merit as though the existence of a liability of a certain population group to scarlet fever should be measured by comparing the incidence rate of all exanthematous diseases in the test group with the incidence rate of all exanthematous infectious diseases in the general population.

Likewise, it would not occur to any competent epidemiologist to determine the existence of a pulmonary tuberculosis hazard in a population group by ascertaining the general pulmonary infectious disease rate of a test population and by comparing it with that of the population in general.

This statistical procedure not only is methodologically incorrect but might readily lead to incorrect and misleading results. Since the general class "infectious diseases" has been separated into a large number of individual disease entities, well characterized by etiologic and symptomatic criteria, the pursuit of such epidemiologic fallacies is not customary in the infectious disease field, although it is still common in the field of cancer.

There is as slight an "intrinsic" liability to infectious diseases as there is to cancer. Both groups of diseases are caused by specific and diverse pathogenic agents, which show marked quantitative and qualitative variations in their spectrum in different population groups, at different times, and under different environmental conditions. Without these agents present, there is no disease. It is obvious from these considerations and facts that the existence of identical attack rates of cancer of a certain site for different population groups does not permit the conclusion that in these groups the various carcinogenic agents which may produce cancer of a particular organ are present and active in the same quantitative and qualitative relations. It is theoretically possible that the cancers at a certain site may be elicited in one group by one single carcinogenic agent, while they are caused in another by a great variety of such agents. The actual existence of such conditions is strongly suggested by some experiences made in the field of occupational cancer whenever the attack rate of a particular cancer approaches 100% of the population at risk. It is readily conceivable that similar conditions might prevail in less circumscribed population groups even in the presence of lower attack rates, whenever one particular environmental carcinogen dominates for some reason, such as one single type of industrial operation, or the activity of the environmental carcinogenic spectrum for a circumscribed population group.

There are, moreover, no sound and rational biologic grounds to expect, even under such special conditions of exposure to a carcinogenic agent, the development of cancers in all members of such population groups. Individual differences in the type and intensity of exposure to the agent, as well as in the degree of constitutional susceptibility, exert their controlling influence on attack rates to cancer, as they do for those of infectious disease endemics and epidemics. Attack rates approaching 100% of the exposed population are exceptions for both types of environmental diseases. Such end-results are, moreover, unlikely for chronic diseases, which apparently develop several distinct reaction phases in the host organism, characterized by different types of reaction products. It is thus conceivable that cancer as the final reaction product of a long chain of preceding events in the tissues specifically stimulated is not an obligatory outcome of such developments and therefore may be compared in this respect with the irregular occurrence of metasyphilitic manifestations (dementia paralytica, tabes) representing the fourth-stage signs in chronic syphilis.

Because of the lack of adequate and reliable etiology-specific criteria of human cancers, there is a tendency to mistake coincidental statistical relations to environmental agents for causal relations. It seems to be wise, before such doubtful statistical conclusions are drawn, to check the statistical evidence against the available

data of the total symptomatic cancer pattern. Any inconsistencies discovered by such a comparison should create serious doubts as to the validity of the causal statistical conclusions. The presently raging controversy as to the existence of causal connections between cigarette smoking and the development of lung cancer offers a striking illustration of this point. Conceding, for the matter of argument, that there exists a universally demonstrable and significant statistical association between these two events (which, however, in fact does not invariable exist), the following observations militate against the presence of causal relations: 1. The liability to lung cancer does not differ for persons who inhale and those who do not inhale, although there must be a marked difference in the degree of exposure of the lungs to smoke between the two groups. 2. The statistical absence of an abnormally high incidence of respiratory irritation symptoms, such as chronic cough, among smokers, in comparison with nonsmokers, does not agree with the known clinical facts in this matter. Smoker's cough is a well-established clinical phenomenon. 3. If the smoking of cigarettes represents a more powerful respiratory cancer hazard than that of pipes or cigars, as asserted, one should expect that there would exist an excessive liability to cancer not only of the lung, but also of the oral cavity, for cigarette smokers, because the tissues of the mouth are the sites of first contact for all three types of smokers. In fact, one would be likely to assume, on the basis of experiences made with occupational cancer hazards, that the liability to cancer of the oral cavity would be higher in cigarette smokers than in pipe and cigar smokers. The statistical evidence, on the other hand, shows that a reverse statistical relation is observed. 4. The start in the rise in lung cancer incidence preceded by two decades the widespread adoption of the cigarette-smoking habit (start of rise about 1900; latent period of lung cancer from cigarette smoking 30 to 40 years). 5. These marked variations in the incidence rate of lung cancer in different geographic regions, in population groups of similar environmental background (metropolitan areas), between urban, industrialized, and rural population groups, and in sex ratio in different lung cancer studies (male-to-female ratio ranging from 2:1 to 25:1); and a considerable number of potent occupational and environmental carcinogens affecting the respiratory system of wide distribution and related in their occurrence and intensity of action to the development of modern industrialization have been demonstrated.

In any valid epidemiologic analysis, this evidence deserves serious consideration and cannot simply be disregarded. Competent epidemiologic investigations in environmental cancer require a well-balanced consideration of both statistical and medical evidence. Only when data from the two sources coincide to a wide degree is it permissible to draw definite conclusions, unless, exceptionally, the medical evidence alone is sufficiently characteristic to establish causal relations between exposure factors and the development of cancer. Statistical and epidemiologic methods now in use for the study of human cancer are more suitable for finding attack rates and age, sex, and race selection than for establishing definite relations to specific causal agents, although such data may provide important supporting evidence on the causal aspects obtained by medical and experimental investigations.

If we contemplate the fact that, according to official information, health hazards exist for 35,000 persons exposed to asbestos dust, 33,000 workers having contact with arsenicals, 28,000 employees exposed to benzene, 240,000 workers inhaling various types of metal dust, and 2,500,000 workers having cutaneous, respiratory,

and ingestive contact with various combustion and distillation products of coal tar, pitch, creosote, soot, and petroleum oils, fuels, and greases—to name a few groups of workers with potentially carcinogenic contacts—the problem of the study and future control of occupational and environmental cancer hazards is impressive indeed. It will tax our scientific and technologic ingenuity, our courage, our moral sensitivity, and our economic resources to a not small degree, if we shall hope to achieve a reasonable amount of control.

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CORRECTION

The type of the first page of the article, "Metastases of Primary Urinary Bladder Carcinoma Invading the Prostate," by Drs. Otto Saphir and H. J. Schwars, in the September issue, accidentally became jumbled. The first four paragraphs should read as follows:

Metastases from primary carcinomas of the urinary bladder are considered rare and are usually seen only late in the disease.[1] We have observed a number of such instances which we considered early cases and in which there were metastases to various organs, while in other, advanced cases the carcinoma was localized in the urinary bladder and there were no metastases. This investigation was undertaken to determine whether the presence of metastases was dependent upon the degree of malignancy or upon other factors.

This study is based on autopsies on 61 primary carcinomas of the urinary bladder, of which 46 were in males and 15 were in females. The carcinoma was either a transitional cell carcinoma or a combination of transitional cell and squamous cell carcinoma. There was only one mucin-secreting adenocarcinoma.

Of the 46 cases in males, the primary carcinoma arose in the region of the trigone in 24 instances, in 10 at the dome of the bladder, and in 6 cases from the posterior wall, without involvement of the trigone. In five other cases the entire wall of the bladder was involved and the exact origin of the tumor could not be determined. In one case the tumor consisted of multiple papillary excrescences. The cases were graded according to Ash[2] on the basis of pleomorphism and mitoses of the cells, irrespective of their invasiveness. In 3 instances the carcinoma was Grade 1, in 20 Grade 2, in 11 Grade 3, and in 12 Grade 4. In 23 of the 46 males, the carcinoma had invaded the prostate. This was verified microscopically in every instance: Seven of these carcinomas were Grade 2; 6, Grade 3, and 10, Grade 4.

Table 1 is included to show the type and grade of malignancy, the location of the primary carcinoma, and the site of metastases of the 23 carcinomas of the urinary bladder which had invaded the prostate. Because of autopsy restrictions, complete examination of bones for metastases could not be performed. Only those few cases are listed in which osseous metastases were obvious in ribs, vertebrae, or pelvic bones.

News and Comment

American Cancer Society Awards Research Grants.—American Cancer Society institutional and special purpose research grants for 1954-1955 are as follows:

Boston University: C. S. Keefer. Chemical structure and function of human malignant tumors.

Brown University: J. W. Wilson. Histophysiology, histopathology, and cytology of liver and skin.

University of Buffalo: S. Kimball. Institutional cancer research program of the University of Buffalo.

University of California: D. A. Wood. Cancer research in action.

University of Chicago: L. T. Coggeshall. Cause, diagnosis, and treatment of cancer.

University of Colorado: R. C. Lewis. Cellular biology, tissue growth, and endocrinology.

Columbia University: W. C. Rappleye. Research studies of cancer.

Detroit Institute of Cancer Research and Wayne University: W. L. Simpson and G. H. Scott. Integrated studies of experimental and clinical cancer.

Emory University: A. P. Richardson. Basic research in cancer.

George Washington University: W. A. Bloedorn. Clinical and research program.

Harvard University: J. C. Aub. Research in growth and cancer.

Indiana University Medical Center: E. A. Lawrence. Metabolic changes in natural and induced resistance to malignant neoplasms.

Institute for Cancer Research, Philadelphia: S. P. Reimann. Cancer research through application of various techniques.

State University of Iowa: N. B. Nelson. Fundamental studies in cellular physiology.

Johns Hopkins University: W. W. Scott. Studies on the etiology and treatment of tumors.

University of Kansas: R. E. Stowell. Physical, chemical, structural, and functional changes associated with cancer.

Massachusetts General Hospital: P. Zamecnik. Basic science and clinical investigations into cause and cure of cancer.

University of Michigan: A. C. Furstenberg. Integrated studies of the nature, detection, and cure of cancer.

University of Minnesota: H. S. Diehl. Institutional cancer research program.

New York University, Bellevue Medical Center: G. H. Twombly. Cancer research at New York University Medical School with clinical research at Bellevue.

Ohio State University: C. A. Doan. Coordinated institutional cancer research program.

Oklahoma Medical Research Foundation: C. D. Kochakian. Clinical and basic research in normal and abnormal growth.

University of Pennsylvania: E. P. Pendergrass. Cancer research program.

University of Rochester: J. J. Morton and E. H. Keutmann. Tumor host relationships.

Roscoe B. Jackson Memorial Laboratory: Exploration of research leads in growth and cancer.

Rutgers University: J. B. Allison. Dynamic state of cancerous and other tissues.

Colonel Hullinghorst Takes New Position.—Col. Robert L. Hullinghorst, Medical Corps, Acting Chief of the Research Division, Armed Forces Institute of Pathology, has taken over his new position as Assistant Commandant of the Army Medical Service Graduate School at Walter Reed Army Medical Center, Washington, D. C. Colonel Hullinghorst was awarded the Legion of Merit for services in World War II.

Dr. Mellon Retires.—Dr. Ralph R. Mellon, of Pittsburgh, has recently retired as Director of the Institute of Pathology of Western Pennsylvania Hospital. Dr. Mellon has been associated with the Institute since 1927.

Course on Surgical Pathology.—University of California Extension and the Division of Postgraduate Medical Education at the University of California at Los Angeles offer a course in general surgical pathology to graduates of approved medical schools on Saturdays, 9 a. m. to 1 p. m., from Nov. 13, 1954, to Jan. 22, 1955, in Room 14-111 of the School of Medicine on the university campus. The fee for the course is $80. Application or request for information concerning the course should be made to Dr. Thomas H. Sternberg, U. C. L. A. Medical Center, Los Angeles 24.

Augustus B. Wadsworth Lecture.—The Fifth Augustus B. Wadsworth Lecture of the New York State Department of Health was given on Oct. 28, 1954, by Dr. Fred W. Stewart, of the Memorial Center for Cancer and Allied Diseases, New York. Dr. Stewart talked on the subject "Wadsworth and Ewing: Problems That Would Interest Them Today."

Dr. Wartman Acts As Symposium Moderator.—Dr. William B. Wartman, Chairman of the Department of Pathology of Northwestern University Medical School, served as moderator in a symposium dealing with diseases of the myocardium presented at the Chicago Academy of Sciences on Oct. 26, 1954.

Lecture on Rheumatic Fever.—The Robert A. Black Memorial Lecture was given at the La Rabida Jackson Park Sanitarium, Chicago, on Oct. 15, 1954, by Dr. Eleanor M. Humphreys, Professor of Pathology, University of Chicago. Her subject was "Rheumatic Fever—Observations and Perspectives."

Dr. Von Glahn Retires.—Dr. William C. Von Glahn, Chairman of the Department of Pathology at the New York University College of Medicine, New York, has retired with the title of Professor Emeritus. He has been succeeded by Dr. Lewis Thomas, formerly of the University of Miami School of Medicine, Coral Gables, Fla.

Inter-Society Cytology Council.—The Second Annual Meeting of the Inter-Society Cytology Council will be held on Nov. 12 and 13, 1954, at the Hotel Statler, Boston. Among the papers scheduled for the morning session of Nov. 12, James W. Reagan, Cleveland, chairman, will be Observations with the Electron Microscope on the Structure of the Cell Surface, by D. W. Fawcett, Boston; Applications of New Cytochemical Methods to Cancer Cytology, by M. J. Kopac, New York; A Multidimensional Analysis of Some Quantitative Characteristics of Exfoliated Cells in Papanicolaou Smears, by W. E. Tolles, Mineola, N. Y., and Vaginal Cytology After Castration and Adrenalectomy, by G. L. Wied, M. E. Davis, and C. Huggins, Chicago.

The afternoon program will consist of papers on Prognosis in Cancer of the Cervix by Histologic and Cytologic Techniques, with Arthur T. Hertig, Boston, acting as chairman.

On Nov. 13, from 9:00 to 12:00, with Eemerson Day, New York, as chairman, papers on New Developments in Cytology will be presented.

In the afternoon there will be a round-table discussion of Carcinoma in Situ of the Uterine Cervix, with John R. McDonald, Rochester, Minn., chairman.

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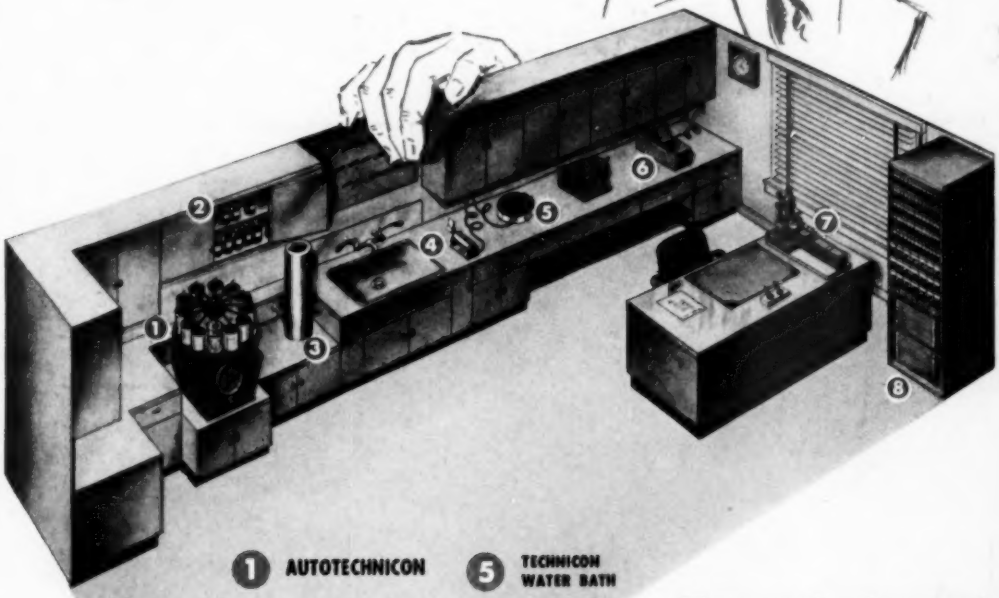

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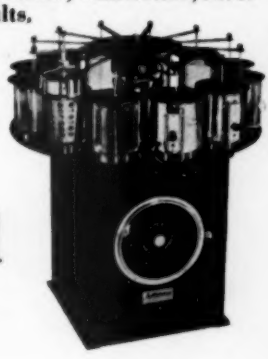

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